TroCCAP

Tropical Council for Companion Animal Parasites

Guidelines for the diagnosis, treatment and control of canine endoparasites in the tropics.

Second Edition March 2019.

Disclaimer

The guidelines presented in this booklet were independently developed by members of the Tropical Council for Companion Animal Parasites Ltd.

These best-practice guidelines are based on evidence-based, peer reviewed, published scientific literature. The authors of these guidelines have made considerable efforts to ensure the information upon which they are based is accurate and up-to-date.

Individual circumstances must be taken into account where appropriate when following the recommendations in these guidelines.

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Contents

General Considerations and Recommendations

Diagnosis

- Dogs should be tested for gastrointestinal parasites at least once every 3 months to monitor the efficacy of parasite control regimes and owner compliance.
- Standard or modified faecal flotation using a solution with specific gravity (S.G.) generally between 1.18 to 1.25 is recommended for the diagnosis of the majority of gastrointestinal parasites of dogs.
- Clinical signs might occur prior to shedding of parasite stages in the faeces, in which case, history and clinical signs should guide treatment decisions.
- Diagnosis of gastrointestinal parasitic infections may be complicated by an absence or intermittent shedding of eggs/ larvae in the faeces, even in symptomatic cases. Testing three or more samples, on alternate days, may increase the probability of finding diagnostic stages in the faeces.
- Blood or buffy coat smears from animals suspected of haemoparasitic infections should be performed using capillary blood collected via ear-tip or outer lip margin.
- Vector-borne parasites can be detected using various specific laboratory methods, some being available as in-clinic commercial tests.
- In some cases, ancillary tests (e.g. blood counts, urinalysis, x-ray, and echocardiography) should be conducted to better guide treatment and management of the patient. In some instances, imaging tools may also be helpful to confirm the diagnosis; e.g. echocardiography may reveal the presence of heartworms in the right ventricle and computed tomography scan may indicate the presence of *Onchocerca lupi* in the retrobulbar space.

Treatment

- TroCCAP does not recommend the off-label (extra-label) use of drugs for controlling parasites in dogs. In cases where a registered product is not available (e.g. heartworm adulticides are not available in many heartworm endemic countries), the off-label use of alternative protocols (e.g. slow-killing therapy for heartworm infections) may be the only option.
- The decision of using off-label drugs or protocols should rely on the recommendation of the veterinary practitioner in charge. The veterinarian should apply caution when recommending off-label use of drugs and closely monitor the dog for any unexpected adverse events; the responsibility for any adverse event related to the off-label use of drugs and doses lies with the prescribing veterinarian.
- Generic brands are often available and more accessible. However, veterinarians should be cautious when prescribing generic products. TroCCAP advocates the use of products for which information on efficacy, safety, and quality control is available from the manufacturer.
- Caution should be applied when using off-label macrocyclic lactones, especially in dogs with the ABCB1 (MDR1) gene mutation (e.g. Collies). Toxicity is also dependent on dose and route of administration, with topical application being generally better tolerated than oral and injectable ones.

- Care should be taken to minimize the risk of parasite transmission and morbidity, especially in puppies, by improving nutrition, environmental hygiene, and avoiding overcrowding and other stressors.
- Anthelminthic therapy should be combined with supportive care (e.g. electrolyte fluid therapy, blood transfusion, iron supplementation, and high protein diet) where necessary.
- All dogs and where applicable, cats, should be treated at the same time when residing in the same household or kennel.
- Blood donor dogs should be in optimal health and blood screened using PCR and serological tests to rule out the presence/exposure to parasites that can be transmitted by blood transfusion, including *Babesia* spp., *Leishmania infantum* and *Hepatozoon canis*. Further information on blood transfusions can be found at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4913655/pdf/JVIM-30-015.pdf
- Crystalline fluid therapy should be avoided in severely anaemic patients unless the patient is significantly dehydrated. In this case, pack-cell volume must be closely monitored.

Prevention and control

- Puppies should be dewormed fortnightly until 8 weeks of age, preferably with a product with activity against adults and immature worms (e.g., moxidectin, emodepside) and then monthly thereafter. Adults dogs should be dewormed monthly. More frequent deworming in adult dogs might be required in cases of heavy burdens or when adulticide therapy only is used.
- Prompt, daily removal and disposal of faeces is recommended.
- Concrete and paved surfaces may be soaked in disinfectants (e.g. 1% sodium hypochlorite solution (bleach),10% iodine, 5% potassium permanganate, chloroxylenol or chlorocresol) to kill or at least reduce the viability of helminth eggs and larvae.
- Disinfection of gravel, loam surfaces or lawns with sodium borate (5 kg/m^2) will kill larvae, but will also destroy vegetation.
- Do not feed raw meat or allow dogs to hunt as many animals, birds and reptiles act as intermediate or paratenic hosts for some gastrointestinal and lung parasites.

Public health considerations

- Several parasites of dogs (e.g. *Ancylostoma* spp., *Toxocara canis*, *Echinococcus* spp., *Leishmania infantum* and certain filariae) are zoonotic and their control is also important from a public health perspective.
- Veterinarians and public health workers should educate dog owners regarding the potential risks of improper parasite control in dogs. Many parasites are zoonotic and may affect especially young children and immunocompromised individuals.
- Veterinarians should also advocate good hygienic practices (e.g. hand washing, wearing footwear while outdoors, and prompt removal of dog faeces) for dog owners to minimize the risks of zoonotic parasite transmission.

Gastrointestinal Parasites

Hookworms (*Ancylostoma* spp., *Uncinaria stenocephala*)

Hookworms are nematodes that infect domestic and wild canids, felids and primates. Dogs become infected with ensheathed third-stage larvae via the percutaneous (skin), oral or trans-mammary routes (*Ancylostoma caninum* only). They are zoonotic.

Parasite: *Ancylostoma caninum*, *Ancylostoma ceylanicum*, *Ancylostoma braziliense*, *Uncinaria stenocephala*

Common name: Hookworm

Host: Dogs, cats, wild canids and felids, primates (including humans)

Pre-patent period: 2 to 4 weeks depending on site of infection

Location of adults: Small intestine

Distribution: Worldwide

Transmission route: Ingestion of third stage larva (all), percutaneous (all) and via trans-mammary route (*A. caninum* only)

Zoonotic: Yes

Distribution

A. caninum is found in wet and dry regions of the tropics and subtropics. *Ancylostoma ceylanicum* is found in the wet tropics and subtropics of Southeast Asia, China, India, and Oceania. *Ancylostoma braziliense* is found in the wet tropics of Central and South America, Malaysia, Indonesia, and northern Australia. *Uncinaria stenocephala* is usually found in temperate, cooler climates in sub-tropical regions.

Clinical signs

In puppies (as young as 10 days old for *A. caninum*), diarrhoea, often bloody, anaemia, hypoproteinaemia and death may ensue. In older dogs, non-regenerative iron deficiency anaemia may result.

Diagnosis

Detection of strongyle eggs (**Fig 1**) on standard faecal flotation (**SOP 1**) using saturated salt or sodium nitrate solution (S.G. 1.20). Immature worms may still produce clinical disease (i.e. no eggs observed in faeces). In this case, treatment and examination of expelled worms is recommended (**Figs 2 and 3**).

Treatment

For anthelmintic treatment options refer to **Table 1**.
Anthelmintic therapy should be combined with supportive care (e.g. fluid and electrolyte therapy, blood transfusion, iron supplementation, high protein diet), where necessary.

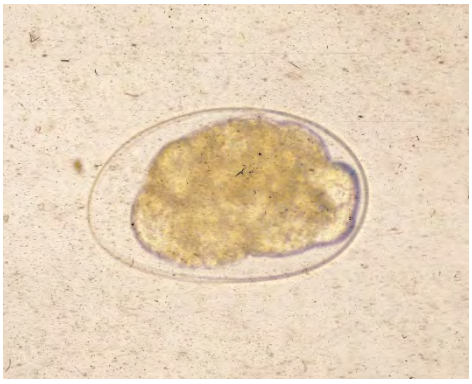

Figure 1 Hookworm egg on faecal flotation. (*Image credit: Dr. R. Traub*)

Figure 2 Buccal capsule of *Ancylostoma caninum* containing three pairs of teeth. (*Image credit: The University of Melbourne parasite image library*)

Figure 3 Buccal capsule of *Ancylostoma ceylanicum* or *Ancylostoma braziliense*, containing a single pair of teeth. (*Image credit: The University of Melbourne parasite image library)*

Table 1 Routes of application, dose and efficacies of commonly utilised anthelmintics against the primary gastrointestinal parasites of dogs.

Anthelmintic	Route	Dose	Hookworm	Roundworm	Whipworm	*Giardia*
Pyrantel pamoate	Oral	5 mg/kg	✓	✓		
Pyrantel embonate	Oral	14 mg/kg	✓	✓		
Pyrantel pamoate /febantel	Oral	5 mg/kg and 15 mg/kg	✓	✓	✓	✓
Emodepside	Oral	0.45 mg/kg	✓	✓	✓	
Oxantel embonate	Oral	55 mg/kg			✓	
Milbemycin*	Oral	0.5 mg/kg	✓	✓	✓	
Moxidectin	Topical	2.5 mg/kg	✓	✓	✓	
Ivermectin	Oral	0.20 mg/kg	✓	✓	✓	
Selamectin	Topical	6 mg/kg	✓	✓		
Fenbendazole	Oral	50 mg/kg for 3 consecutive days[€]	✓	✓	✓	✓
Oxibendazole	Oral	10-20 mg/kg	✓	✓	✓	

*Poor efficacy against *Uncinaria stenocephala*
[€] For treatment of *Giardia* infections, administer for 5 consecutive days

Control

Puppies should be treated with a registered anthelmintic labelled for use in puppies at 2 weeks of age (to prevent vertically acquired infections becoming patent) and then every 2 weeks until 8 weeks of age. Treat the dam at the same time. Following this, dogs should be dewormed fortnightly, or if using moxidectin, then monthly (2.5 mg/kg topically). Refer to **Table 1** for details.

Puppies should be tested for parasites (**SOP 1**) during routine consultations (e.g. vaccinations) and at least every 3 months thereafter to monitor the efficacy of the parasite control regime and owner compliance.

For further control options, refer to the **General Considerations and Recommendations** section.

N.B. Off-label use of anthelmintics that significantly reduce the burden of trans-mammary transmission of *A. caninum* from dam to pups has been described in published literature. These include:

- Spot-on formulation of imidacloprid 10% plus moxidectin 2.5% at day 56 of gestation [1].
- Fenbendazole 50mg/kg daily, from day 40 of gestation to 14 days post-whelping [2].
- Ivermectin intramuscular (300 µg/kg) on days 45 and 55 post conception [3].

Public health considerations

All animal hookworms are zoonotic and may cause cutaneous larva migrans in people. Penetration of the ensheathed larvae produce a mild, self-limiting pruritic rash called 'ground itch'. *Ancylostoma braziliense* may produce 'creeping eruptions', highly pruritic mobile linear or serpent-like dermal lesions. In Asia and Oceania, dogs act as reservoirs for *A. ceylanicum* [4], which produces patent (egg-positive) symptomatic hookworm disease in humans. Non-patent immature *A. caninum* worms may cause eosinophilic enteritis in humans. Most *A. caninum* infections in humans appear asymptomatic.

References

[1] Kramer F, Hammerstein R, Stoye M, Epe C. Investigations into the prevention of prenatal and lactogenic *Toxocara canis* infections in puppies by application of moxidectin to the pregnant dog. *J Vet Med B Infect Dis Vet Public Health*. 2006;53:218-223.

[2] Burke TM, Roberson EL. Fenbendazole treatment of pregnant bitches to reduce prenatal and lactogenic infections of *Toxocara canis* and *Ancylostoma caninum* in pups. *J Am Vet Med Assoc*. 1983;183:987-990.

[3] Stoye M, Meyer O, Schnieder T. The effect of ivermectin on reactivated somatic larva of *Ancylostoma caninum* Ercolani 1859 (Ancylostomidae) in the pregnant dog. *Zentralbl Veterinarmed*. 1989;36:271-278.

[4] Traub, RJ. *Ancylostoma ceylanicum* – a re-emerging but neglected parasitic zoonosis. *Int J Parasitol*. 2013;43:1009-1015.

Roundworms (*Toxocara canis*, *Toxascaris leonina*)

Roundworms are nematodes that can infect domestic and wild canids and felids. Animals become infected when they ingest eggs containing infective larvae. *Toxocara canis* primarily affects puppies, producing signs of enteritis, and it is zoonotic.

Parasite: *Toxocara canis*, *Toxascaris leonina*

Common name: Roundworms

Host: Dogs, cats (*T. leonina* only)

Location of adults: Small intestine

Distribution: Worldwide

Transmission route: Oral (ingestion of eggs with infective larvae), transplacental and trans-mammary (*T. canis* only)

Zoonotic: Yes (*T. canis* only)

Distribution

Worldwide.

Clinical signs

In neonates and puppies, heavy infections via the transplacental route may result in pneumonia and acute death owing to enteritis and gastrointestinal blockage as early as 10 days of age. Heavy burdens with *T. canis* in pups may produce ill thrift, stunting, abdominal discomfort (pups adopt a straddle-legged posture and a pot-bellied appearance), anorexia, diarrhoea and vomiting (adult worms may be expelled). Occasional gastrointestinal obstruction (**Fig 1**) and death may result. *Toxascaris leonina* infection is usually asymptomatic.

Figure 1 Adult worms of *Toxocara canis* exposed within the small intestines of a dog. (*Image credit: The University of Melbourne parasite image library*)

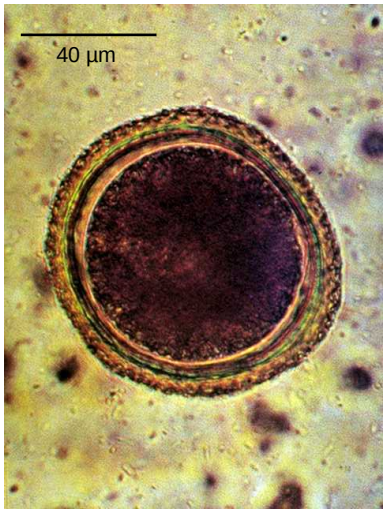

Figure 2 *Toxocara canis* egg on faecal flotation showing pitted surface. (*Image credit: Dr. R. Traub*)

Figure 3 *Toxascaris leonina* eggs on faecal flotation showing smooth surface. (*Image credit: Dr. R. Traub*)

Diagnosis

Detection of thick-shelled (pitted for *Toxocara* (**Fig 2**), smooth for *Toxascaris* (**Fig 3**)) eggs on standard faecal flotation (S.G. 1.20) (**SOP 1**). Immature worms may still produce clinical disease in puppies. Therefore, the absence of eggs in faeces does not rule out infection. In this case, treatment and examination of expelled worms is recommended.

Treatment

For anthelmintic treatment options refer to **Table 1**.
Anthelmintic therapy should be combined with supportive care (e.g. fluid and electrolyte therapy) where necessary.

Table 1 Routes of application, dose and efficacies of commonly utilised anthelmintics against the primary gastrointestinal parasites of dogs.

Anthelmintic	Route	Dose	Hookworm	Roundworm	Whipworm	*Giardia*
Pyrantel pamoate	Oral	5 mg/kg	✓	✓		
Pyrantel embonate	Oral	14 mg/kg	✓	✓		
Pyrantel pamoate /febantel	Oral	5 mg/kg and 15 mg/kg	✓	✓	✓	✓
Emodepside	Oral	0.45 mg/kg	✓	✓	✓	
Oxantel embonate	Oral	55 mg/kg			✓	
Milbemycin*	Oral	0.5 mg/kg	✓	✓	✓	
Moxidectin	Topical	2.5 mg/kg	✓	✓	✓	
Ivermectin	Oral	0.20 mg/kg	✓	✓	✓	
Selamectin	Topical	6 mg/kg	✓	✓		
Fenbendazole	Oral	50 mg/kg for 3 consecutive days€	✓	✓	✓	✓
Oxibendazole	Oral	10-20 mg/kg	✓	✓	✓	

*Poor efficacy against *Uncinaria stenocephala*
€ For treatment of *Giardia* infections, administer for 5 consecutive days

Control

Puppies should be treated with a registered anthelmintic labelled for use in puppies at 2 weeks of age (to prevent vertically acquired infections becoming patent) and then every 2 weeks until 8 weeks of age. Treat the dam at the same time. Following this, dogs should be dewormed monthly. Refer to **Table 1** for details on recommended frequency of administration for individual anthelmintics. For further control options, refer to the **General Considerations and Recommendations** section.

In adult dogs, there is a high probability that *T. canis* infection will result in somatic migration with larvae in the tissues. Therefore, an absence of *T. canis* eggs in adult dogs does not rule out infection, as arrested larvae may re-activate during pregnancy to infect pups *in-utero*.

Off-label use of anthelmintics that significantly reduce the burden of vertical and trans-mammary transmission of *T. canis* from dam to pups has been described in published literature. These include:

- Topical selamectin applied at 6 mg/kg at 40 and 10 days pre-parturition and 10 and 40 days post-whelping [1].
- Fenbendazole 50 mg/kg daily, day 40 to 14 days post-whelping [2].
- Ivermectin SC administered at 300 µg/kg body weight on days 0, 30 and 60 plus 10 days post whelping [3].

Public health considerations

Ingestion of embryonated *T. canis* eggs in the environment may produce covert, ocular or visceral larva migrans. Children are most at risk owing to their behaviour. Once ingested the larvae undergo somatic migration to organs such as the liver, lungs, brain and eye. Such migration may be asymptomatic or alternatively, larval migration can lead to an eosinophilic inflammatory response producing clinical symptoms such as abdominal pain, fever, hepatomegaly and cough. Symptoms are usually self-limiting, but may lead to serious complications if there is neurological or cardiac involvement. *Toxocara canis* larvae may enter the eye and its vasculature causing blindness or decreased vision due to retinochoroiditis, optic neuritis and endophthalmitis.

References

[1] Payne-Johnson M, Maitland TP, Sherington J, Shanks DJ, Clements PJ, Murphy MG, McLoughlin A, Jernigan AD, Rowan TG. Efficacy of selamectin administered topically to pregnant and lactating female dogs in the treatment and prevention of adult roundworm (*Toxocara canis*) infections and flea (*Ctenocephalides felis felis*) infestations in the dams and their pups. *Vet Parasitol.* 2000;91:347-358.

[2] Burke TM, Roberson EL. Fenbendazole treatment of pregnant bitches to reduce prenatal and lactogenic infections of *Toxocara canis* and *Ancylostoma caninum* in pups. *J Am Vet Med Assoc*. 1983;183:987-990.

[3] Payne PA, Ridley RK. Strategic use of ivermectin during pregnancy to control *Toxocara canis* in greyhound puppies. *Vet Parasitol.* 1999;85:305-312.

Whipworm (*Trichuris vulpis*)

Trichuris vulpis is a whipworm of dogs, also found in foxes and coyotes. Heavy infections may produce signs of large bowel diarrhoea. Dogs become infected when they ingest infective eggs.

Parasite: *Trichuris vulpis*

Common name: Whipworm

Host: Dogs

Pre-patent period: 11 weeks

Location of adults: Cecum and colon

Distribution: Worldwide

Transmission route: Oral (ingestion of embryonated eggs)

Zoonotic: No

Distribution

Worldwide.

Clinical signs

Light whipworm infections are usually asymptomatic. Heavy infections, even in adult animals can produce clinical signs of large bowel diarrhoea (e.g. tenesmus) and faeces may contain mucous and fresh blood. Anorexia, weight loss, colic and anaemia may occur. Some cases have mimicked Addison's disease (primary adrenal insufficiency and hypoadrenalism).

Diagnosis

Because of the long pre-patent period of 10-12 weeks, *T. vulpis* eggs are not commonly found in the faeces of puppies. Dogs however, may show clinical signs before eggs are shed in faeces. Diagnosis is by visualisation of the characteristically bi-plugged, thick-shelled egg (**Fig 1**) on centrifugal faecal flotation (**SOP 2**) using a flotation solution with a S.G. of 1.25 e.g. sugar solution. Alternatively, if a centrifuge is not available, a standard faecal flotation (**SOP 1**) is recommended (S.G. 1.20). Adults have a characteristic 'whip' shaped body with a long thin anterior end embedded in the mucosa and a stout posterior end, which is free in the lumen (**Fig 2**).

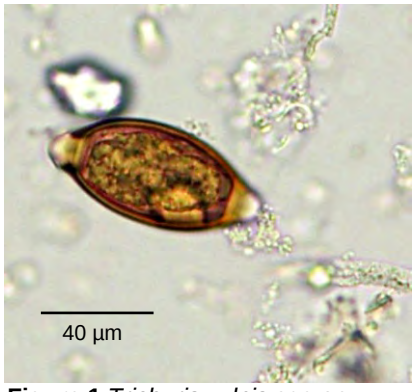

Figure 1 *Trichuris vulpis* egg on faecal flotation. (*Image credit: Dr. T. Inpankaew*)

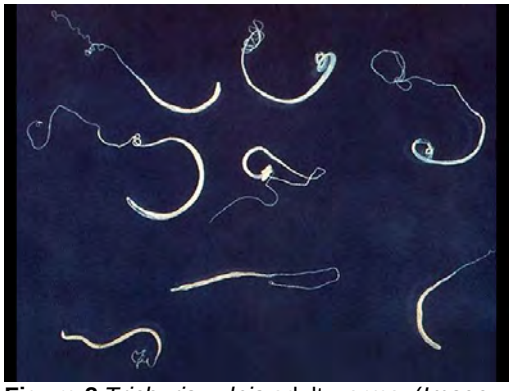

Figure 2 *Trichuris vulpis* adult worms. (*Image credit: The University of Melbourne parasitology image library*)

Treatment

For anthelmintic treatment options refer to **Table 1**.
Anthelmintic therapy should be combined with supportive care (e.g. fluid and electrolyte therapy) where necessary.

Table 1 Routes of application, dose and efficacies of commonly utilised anthelmintics against the primary gastrointestinal parasites of dogs.

Anthelmintic	Route	Dose	Hookworm	Roundworm	Whipworm	*Giardia*
Pyrantel pamoate	Oral	5 mg/kg	✓	✓		
Pyrantel embonate	Oral	14 mg/kg	✓	✓		
Pyrantel pamoate /febantel	Oral	5 mg/kg and 15 mg/kg	✓	✓	✓	✓
Emodepside	Oral	0.45 mg/kg	✓	✓	✓	
Oxantel embonate	Oral	55 mg/kg			✓	
Milbemycin*	Oral	0.5 mg/kg	✓	✓	✓	
Moxidectin	Topical	2.5 mg/kg	✓	✓	✓	
Ivermectin	Oral	0.20 mg/kg	✓	✓	✓	
Selamectin	Topical	6 mg/kg	✓	✓		
Fenbendazole	Oral	50 mg/kg for 3 consecutive days[€]	✓	✓	✓	✓
Oxibendazole	Oral	10-20 mg/kg	✓	✓	✓	

*Poor efficacy against *Uncinaria stenocephala*
[€]For treatment of *Giardia* infections, administer for 5 consecutive days

Control

Repeat treatments in 2.5-3 months to destroy developing larvae as they mature.
For further control options, refer to the **General Considerations and Recommendations** section.

Public health considerations

None.

Intestinal Threadworm (*Strongyloides stercoralis*)

Strongyloides spp. infect dogs, cats and humans. Dogs become infected when they ingest infective larvae through mammary milk or when these larvae actively penetrate into the dogs' skin.

Parasite: *Strongyloides stercoralis* (syn. *Strongyloides canis*)

Common name: Intestinal threadworm

Host: Dogs, humans, cats

Pre-patent period: 6-10 days; autoinfection possible

Location of adults: Small intestine

Distribution: Worldwide

Transmission route: Percutaneous, trans-mammary and auto-infection [i.e., rhabditiform larvae become infective filariform larvae, which can penetrate either the intestinal mucosa (internal autoinfection) or the skin of the perianal area (external autoinfection)]

Zoonotic: Yes

Distribution

Worldwide.

Clinical signs

Most dogs are asymptomatic, developing a strong immunity to infection following transmammary infection and stop shedding larvae within the first 8-12 weeks of life. In young pups, mild and self-limiting watery or mucus diarrhoea may result. Use of corticosteroids or a reduction in immunocompetence predisposes to autoinfection. In these instances, wasting and signs of bronchopneumonia due to migration of auto-infective larvae may be present. Pododermatitis may result from percutaneous penetration of larvae.

Diagnosis

The Baermann technique (**SOP 3**) is the test of choice for larval isolation and identification. Eggs possess first-stage larvae (**Fig 1**), which may be isolated on standard faecal flotation (S.G. 1.20) (**SOP 1**). The first-stage larvae can be recognized via their prominent genital primordium (**Fig 2**) and must be differentiated from larvae of lungworms (**Fig 3**) and

Figure 1 *Strongyloides* eggs containing first stage larvae on faecal flotation. (*Image credit: The University of Melbourne parasitology image library*)

Figure 2 Larva of *Strongyloides* spp. containing a prominent genital primordium (arrow). (*Image credit: The University of Melbourne parasitology image library*)

hookworms. Diagnosis of *Strongyloides* spp. infection is complicated by the fact that larvae may be very low in number or absent from the faeces, even in symptomatic cases. In these cases, faeces can be tested multiple times (3 times over the course of 5 to 7 days).

Figure 3 First-stage larva of a canine lungworm containing a 'kink' in the tail. (*Image credit: Dr. R. Traub*)

Treatment

Off-label use of ivermectin at 200 µg/kg, as a single oral dose and fenbendazole 50 mg/kg once daily for 5 days is effective at removing adult worms. Re-test faeces twice at 2- and 4-weeks following treatment and monthly thereafter, for a total period of 6 months. Re-treatment may be necessary in some cases.

Control

In *Strongyloides*-endemic areas, consider testing dogs prior to initiating any immunosuppressive therapy, particularly corticosteroids. Latent intestinal infections can be reactivated when the host is immunocompromised (e.g. iatrogenic, neoplasia) to produce auto-infective larvae, which can cause life-threatening disseminated infection. Infected dogs should be isolated from other animals. For further control options, refer to the **General Considerations and Recommendations** section.

Public health considerations

In humans, clinical signs of *S. stercoralis* infection may range from being asymptomatic to causing gastrointestinal disorders (e.g. abdominal pain, diarrhoea) and cough. Percutaneous penetration of infective larvae may also cause larva currens. In immunocompromised people, autoinfection may result in hyper-infection syndrome, disseminate strongyloidiasis and bacteraemia, which may prove fatal.

Flea Tapeworm (*Dipylidium caninum*)

Dipylidium caninum is a common tapeworm of dogs, foxes and cats. It is transmitted when a dog ingests infected fleas or chewing lice. It is zoonotic.

Parasite: *Dipylidium caninum*

Common name: The flea tapeworm

Hosts: Dogs, foxes, cats, humans

Pre-patent period: 2-3 weeks

Location of adults: Small intestine

Distribution: Worldwide

Transmission route: Oral (ingestion of infected fleas or lice)

Zoonotic: Yes (albeit rare)

Distribution

Worldwide.

Clinical signs

Dipylidium caninum infections are usually asymptomatic. However, the passage of gravid segments through the rectum will cause irritation and the dogs will usually 'scoot' and rub their perineum along the ground. In rare cases, dogs with heavy infections may develop enteritis and/or intestinal obstruction.

Diagnosis

Diagnosis can be made through history (i.e. lack of flea control, lack of deworming with praziquantel) and by detecting proglottids in the faeces, coat, and bedding or around the anus. The proglottids of *D. caninum* can be differentiated from those of *Taenia* spp. by shape and presence of two bilaterally symmetrical genital pores located in the middle of the segment (**Fig 1**). Squashing a gravid proglottid will reveal egg capsules (egg packets) (**Fig 2**). Occasionally, egg capsules are detected by faecal flotation methods but this method is not sensitive.

Figure 1 *Dipylidium caninum* mature proglottid. (*Image credit: The University of Melbourne parasitology image library*)

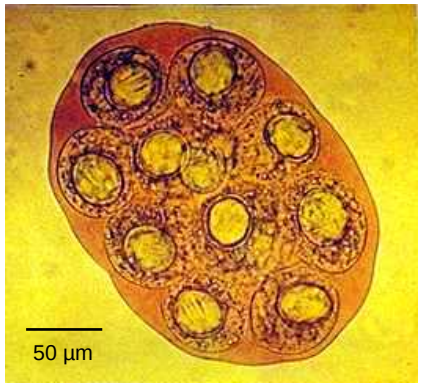

Figure 2 *Dipylidium caninum* eggs within a capsule on faecal floatation. (*Image credit: The University of Melbourne parasitology image library*)

Treatment

Treatment of *D. caninum* infection is by praziquantel at 5 mg/kg every 2 weeks, until flea or lice control is achieved.

Control

Control can be achieved by keeping dogs and cats free of fleas (refer to flea control guidelines) and lice (refer to lice control guidelines).

Public health considerations

D. caninum infection, usually of children, occasionally occurs via ingestion of adult fleas. Children may be asymptomatic or suffer from perianal irritation and/ or light intestinal disturbances. Proglottids may be observed in the faeces or around the perianal area of the child.

Hydatid Tapeworm (*Echinococcus granulosus*)

The parasite is of no clinical significance in dogs, but eggs passed by dogs infect humans and livestock to produce hydatid cysts in visceral organs resulting in significant public health and economic impacts.

Parasite: *Echinococcus granulosus*

Common name: Hydatid tapeworm

Host: Dogs

Pre-patent period: 6-7 weeks

Location of adults: Small intestine

Distribution: Worldwide

Transmission route: Oral (ingestion of fertile hydatid cysts in intermediate host tissue)

Zoonotic: Yes

Distribution

Echinococcus granulosus is distributed globally, but appears to be highly endemic in cooler regions of the sub-tropics (e.g. northern India, Southern Brazil), especially in rural areas where offal is readily accessible to farm and community dogs. It has not been reported in many parts of tropical Africa, Southeast Asia, Central America and the Caribbean.

Clinical signs

Dogs are unlikely to show clinical signs of infection.

Diagnosis

Should be based on the animal's history i.e. access to raw offal. Detection of eggs and proglottids on standard faecal flotation is unreliable as eggs are rarely shed in faeces. When present, eggs are morphologically indistinguishable from eggs of *Taenia* spp. (**Fig 1**). Anthelmintic purgation and examination of adult worms is not recommended due to the zoonotic risk associated with accidental ingestion of *E. granulosus* eggs. Adult worms are minute, measuring 3-9 mm, with a maximum of 3 segments (**Fig 2**).

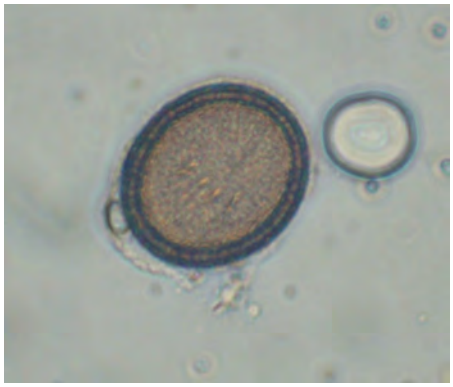

Figure 1 Taeniid egg on faecal floatation. (*Image credit: Dr. R. Traub*)

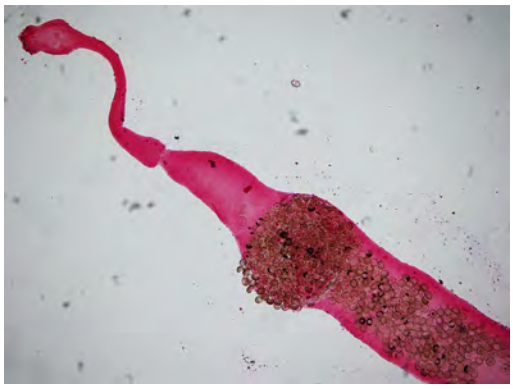

Figure 2 *Echinococcus granulosus* adult worm stained with carmine. (*Image credit: Dr. A. D. Milhalca*)

Treatment

Praziquantel given orally at 5 mg/kg is the drug of choice.

Control

Owners should be strongly encouraged to limit the access of their dog to offal of domestic or wild intermediate hosts (e.g. livestock, horses, camels). In *E. granulosus*-endemic areas, dogs should be treated with praziquantel at 6-weekly intervals. It is imperative that the dog's faeces are promptly disposed of up to 48 hours following treatment. Faeces can be burnt, deep buried or disposed of in a flush latrine or septic tank. Targeting intermediate hosts for cystic echinococcosis control may be undertaken through surveillance and meat inspection at slaughter but also using an infection preventive vaccine (EG95). Awareness campaigns are critical.

Public health considerations

Humans acquire infection by ingesting eggs through direct contact with the dog (eggs stick to dogs' coat and are infective immediately upon defaecation) or via ingesting eggs in contaminated food or water. In humans, infection may be asymptomatic or may reflect impairment of organ function (e.g. brain, lung, heart, liver, etc.) as a result of hydatid cysts (**Fig 3**) putting pressure on adjacent organs. Typically, hydatid disease has a prolonged incubation period of years (cysts take time to grow). Rupture or leakage of a cyst can lead to fatal anaphylactic shock. Treatment is complicated and usually requires a combination of surgical and chemotherapeutic intervention.

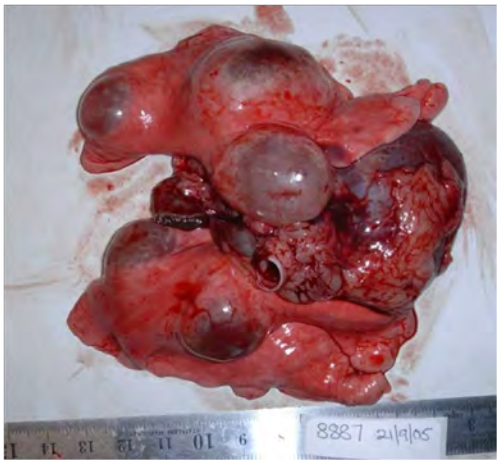

Figure 3 Multiple hydatid cysts in the lungs of a wallaby. (*Image credit: Dr. L. A. Hinds, CSIRO*)

Taenia Tapeworms (*Taenia* spp.)

Tapeworms belonging to the genus *Taenia* are common in dogs that have access to raw carcasses. The primary significance of these canine tapeworms resides in their ability to infect livestock and other animals with larval forms that result in meat condemnation and economic loss at slaughter. *Taenia multiceps* and *T. serialis* are zoonotic.

Parasite: *Taenia hydatigena*, *Taenia ovis*, *Taenia multiceps*, *Taenia pisiformis*, *Taenia serialis*

Common name: Tapeworms

Host: Dogs, foxes, other wild canids

Pre-patent period: 6-8 weeks

Location of adults: Small intestine

Distribution: Worldwide

Transmission route: Oral [ingestion of larval metacestode forms (cysticercus, coenurus) in intermediate host tissue (primarily livestock)]

Zoonotic: Yes (*T. multiceps* and *T. serialis* only)

Distribution

Worldwide.

Clinical signs

Tapeworms are rarely harmful to dogs and most animals are asymptomatic. Heavy infections may cause non-specific abdominal symptoms such as diarrhoea or constipation and abdominal pain accompanied by ill-thrift, and a pot-bellied appearance.

Diagnosis

Proglottids (tapeworm segments) may actively crawl in faeces or around the perianal area of animals (most commonly observed by the owner). Fresh proglottids may be relaxed in water and squashed between two glass slides for morphological examination. Proglottids contain genital pores opening laterally (**Fig 1).** Gravid segments contain typical taeniid eggs (**Fig 2**). Faecal floatation is not recommended for diagnosis as Taeniid eggs are not actively shed in faeces. Eggs of *Taenia* spp. cannot be distinguished from those of *Echinococcus.*

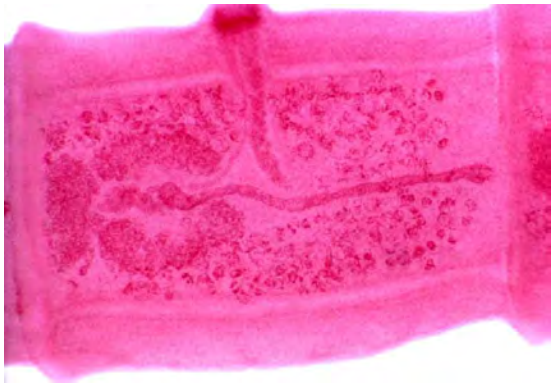

Figure 1 Stained mature proglottid of *Taenia pisiformis.* (*Image credit: M I* (*Spike*) *Walker/Alamy Stock Photo*)

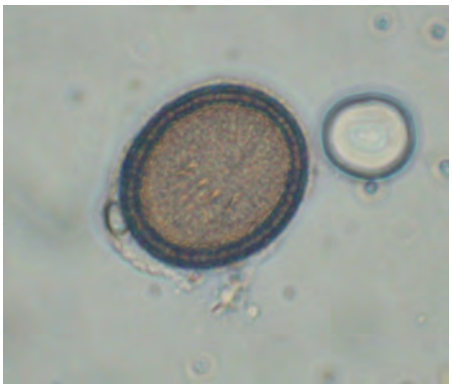

Figure 2 Taeniid egg on faecal floatation. (*Image credit: Dr. R. Traub*)

Treatment

Praziquantel given orally at 5 mg/kg is the drug of choice.

Control

Owners should be strongly encouraged not to feed their dog raw offal or meat of domestic or wild intermediate hosts (e.g. livestock, rabbits). In *Taenia* endemic areas, dogs should be treated with praziquantel at 6-week intervals.

Public health considerations

Ingestion of *T. multiceps* eggs passed in the faeces of canids may result in the larval stage of the tapeworm developing in the central nervous system, eye, subcutaneous or intramuscular tissue of humans, referred to as human coenurosis. Treatment is complicated and usually requires a combination of surgical and chemotherapeutic intervention.

Oesophageal Worm (*Spirocerca lupi*)

Spirocerca lupi is a grossly underestimated and potentially fatal spirurid nematode of domestic and wild canids. Dogs become infected when they ingest intermediate (dung beetles) or transport hosts (e.g. chicken offal, reptiles and rodents).

Parasite: *Spirocerca lupi*

Common name: Oesophageal worm

Host: Canids

Pre-patent period: 5-6 months

Location of adults: Oesophageal and stomach wall

Distribution: Tropical and subtropical regions

Transmission route: Oral (ingestion of intermediate or paratenic hosts)

Zoonotic: No

Distribution

Spirocerca lupi is widely distributed in tropical and subtropical regions of Asia, Oceania, Latin America, the Caribbean, Africa and the Middle East.

Clinical signs

Infected dogs may initially be asymptomatic but can progress to having regurgitation, vomiting, melena, wasting and weight loss as a result of the granulomatous masses in the oesophagus and stomach (**Fig 1**). Aortic migration of larvae may lead to pleuritis resulting in coughing, retching and dyspnoea. Aortic aneurysms (**Fig 2**) may occasionally rupture causing thoracic haemorrhage and sudden death. Fibrous nodules in the oesophagus and stomach may undergo malignant transformation and progress to oesophageal sarcoma with secondary metastases. Hypertrophic osteopathy with front leg periosteal calcification is commonly found associated with a thoracic space occupying lesion in dogs with *S. lupi*-associated neoplasia.

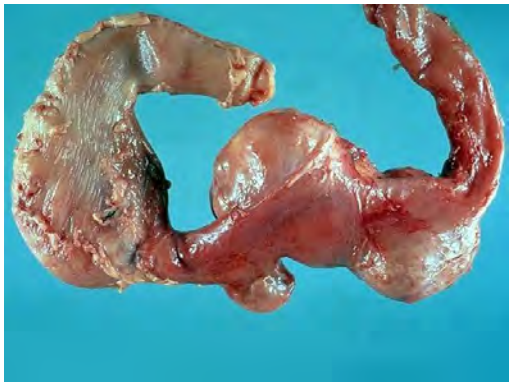

Figure 1 Infection with *Spirocerca lupi* can cause granulomatous masses in the oesophagus and stomach. (*Image credit: The University of Melbourne parasitology image library*)

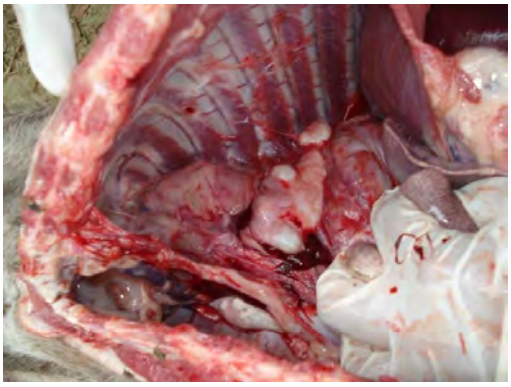

Figure 2 Aortic aneurisms in a dog caused by larvae of migrating *Spirocerca lupi.* (*Image credit: Dr. R. Traub*)

Diagnosis

Faecal egg shedding is intermittent or absent if nodules lack a fistula. Detection of characteristic ellipsoid embryonated eggs (small, 35 × 15 µm) in faeces (**Fig 3**) by standard flotation (**SOP 1)** using a solution with S.G. > 1.20 is optimal. Primary radiological lesions include a mediastinal mass, usually associated with the terminal oesophagus. Spondylitis of the thoracic vertebrae is frequently found on chest radiography. Contrast radiography and computed tomography are helpful additional emerging modalities. Oesophageal endoscopy has a greater diagnostic sensitivity than radiography (**Fig 4**). PCR of faeces has also been shown to be helpful in the diagnosis of *S. lupi* infection [1].

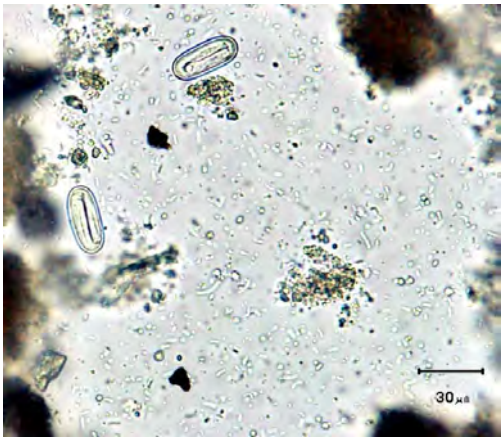

Figure 3 Embryonated *Spirocerca lupi* eggs on faecal flotation. (*Image credit: Dr. T. Inpankaew*)

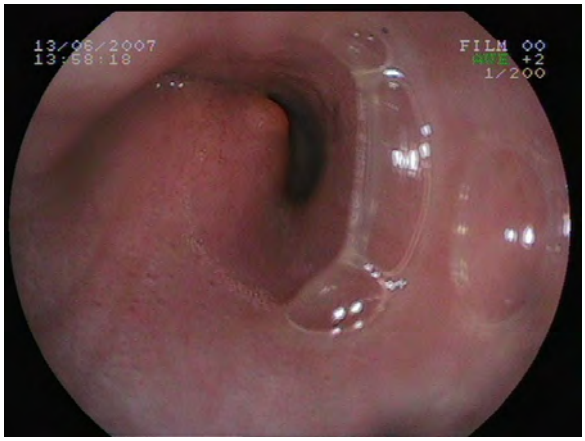

Figure 4 *Spirocerca lupi* granuloma in dog oesophagus viewed on endoscopy. (*Image credit: Dr. G. Baneth*)

Treatment

Treatment is challenging as adults are protected within nodules. Off-label anthelmintic regimes have been shown effective in killing adult worms and reducing the size of granulomas. These include:

- Doramectin 400 µg/kg subcutaneously every 14 days for a total of 6 treatments, followed by 20 additional monthly injections if resolution of nodules is incomplete [2].
- Oral milbemycin 0.5 mg/kg on days 0, 7 and 28 and then monthly [3].
- Topical moxidectin plus imidacloprid weekly for 19 weeks [4].

Food intake may be attempted in an upright standing position in the case of regurgitation due to megaoesophagus.

Control

Monthly application of topical moxidectin plus imidacloprid is approved for use in dogs as a preventative for *S. lupi* infection in Europe.
Dogs should be not allowed to roam outdoors unsupervised or allowed to prey upon paratenic hosts such as rodents, lizards and frogs. For further control options, refer to the **General Considerations and Recommendations** section.

Public health considerations

None.

References

[1] Rojas A, Segev G, Markovics A, Aroch I, Baneth G. Detection and quantification of *Spirocerca lupi* by HRM qPCR in fecal samples from dogs with spirocercosis. *Parasit Vectors.* 2017;10:435.

[2] Lavy E, Aroch I, Bark H, Markovics A, Aizenberg I, Mazaki-Tovi M, Hagag A, Harrus S. Evaluation of doramectin for the treatment of experimental canine spirocercosis. *Vet Parasitol*. 2002;109:65-73.

[2] Kelly PJ, Fisher M, Lucas H, Krecek RC. Treatment of esophageal spirocercosis with milbemycin oxime. *Vet Parasitol.* 2008;156:358-360.

[3] Austin CM, Kok DJ, Crafford D, Schaper R. The efficacy of a topically applied imidacloprid 10 % / moxidectin 2.5 % formulation (Advocate(R), Advantage(R) Multi, Bayer) against immature and adult *Spirocerca lupi* worms in experimentally infected dogs. *Parasitol Res*. 2013;112 Suppl 1:91-108.

Giardia (*Giardia duodenalis*)

Giardia duodenalis is a common protozoan of dogs and a wide range of other hosts including cats, cattle, horses and humans. The primary route of infection is faecal-oral, either through direct, close contact or indirectly via contaminated food and water. Canine giardiasis is a potential zoonosis.

Parasite: *Giardia duodenalis* (syn. *G. lamblia*, *G. intestinalis*)

Common name: Giardia

Host: Many mammalian hosts, including dogs, cats and humans

Pre-patent period: 3 -14 days

Location of trophozoites: Small intestine

Distribution: Worldwide

Transmission route: Oral (ingestion of cysts)

Zoonotic: Yes

Distribution

Worldwide.

Clinical signs

Giardia duodenalis infection is usually asymptomatic, except in young animals. When present, clinical signs include acute or chronic diarrhoea. Affected animals are usually alert and afebrile.

Diagnosis

Zinc sulfate centrifugal flotation (specific gravity 1.18) (**SOP 2**) is the test of choice for the visualization of *Giardia* cysts in faeces (**Fig 1**). Cysts are oval, 10-12 µm long and surrounded by a thin wall. In a diarrheic animal, a fresh faecal smear may reveal motile trophozoites, which have a typical 'falling leaf' motion; however faecal smears can be less sensitive than flotation.

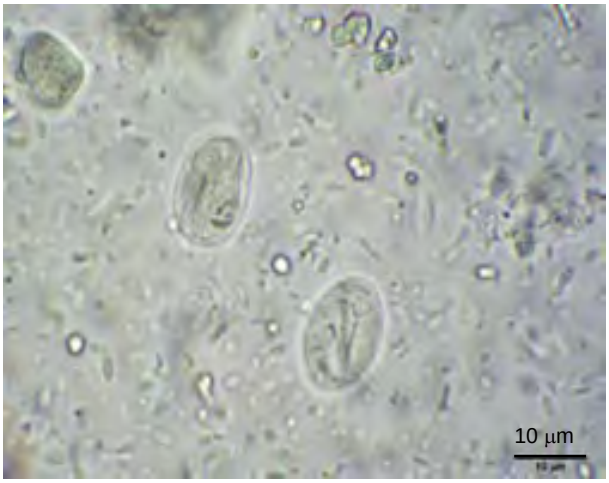

Figure 1 *Giardia* cysts on faecal flotation. (*Image credit: Dr. T. Inpankaew*)

Rapid in-house commercial ELISA-based tests targeting antigens of *Giardia* in canine faeces are available. Alternatively, the sample can be sent to a commercial laboratory for PCR-based detection, where available.

Treatment

Febantel plus pyrantel and praziquantel given daily for 3 days, fenbendazole 50 mg/kg for 5 days and metronidazole 25 mg/kg twice daily for 5-7 days have proven efficacious in the treatment of *Giardia*.

Control

Pregnant females should be tested and treated, and dams bathed before whelping to remove cysts on the coat. Infected animals should be bathed, isolated and moved to a clean, disinfected enclosure once treated. If in a kennel situation, mass treat all animals at the same time. For further control options, refer to the **General Considerations and Recommendations** section.

Public health considerations

Dogs may harbour both dog-specific and zoonotic strains of *Giardia* that cannot be morphologically distinguished. All *Giardia* positive dogs must be suspected of carrying potentially zoonotic strains and owners must be advised on appropriate hygiene practices (see the **General Considerations and Recommendations** section) to minimize the risk of infection.

Coccidia (*Cystoisospora* spp.)

Cystoisospora spp. (=*Isospora* spp.) are apicomplexan protozoa transmitted directly by the faecal-oral route, especially in unhygienic, overcrowded environments. Species harboured by dogs are highly host-specific and a frequent cause of diarrhoea in puppies.

Parasite: *Cystoisospora canis*, *Cystoisospora ohioensis*, *Cystoisospora burrowsi*, *Cystoisospora neorivolta*

Common name: Coccidia

Host: Dogs

Pre-patent period: 5-13 days

Location of adults: Small intestine

Distribution: Worldwide

Transmission route: Oral (ingestion of sporulated oocysts)

Zoonotic: No

Distribution

Worldwide.

Clinical signs

Cystoisospora is most commonly seen in puppies. Common clinical signs include anorexia, vomiting, watery (rarely haemorrhagic) diarrhoea, dehydration and weight loss. Most dogs will develop a strong acquired immunity to infection, shedding only low intensities of oocysts as asymptomatic adults.

Diagnosis

Clinical signs may precede oocyst shedding and, in this case, diagnosis has to be based on history and clinical signs. Oocysts isolated on standard faecal flotation (S.G. 1.20) (**SOP 1**), are unsporulated (**Fig 1**) and develop to infective forms (sporulate) in 2-3 days (**Fig 2**).

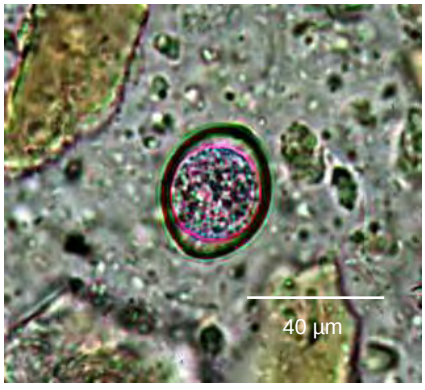

Figure 1 Unsporulated oocyst of *Cystoisospora canis* on faecal flotation. (*Image credit: The University of Melbourne parasite image library*)

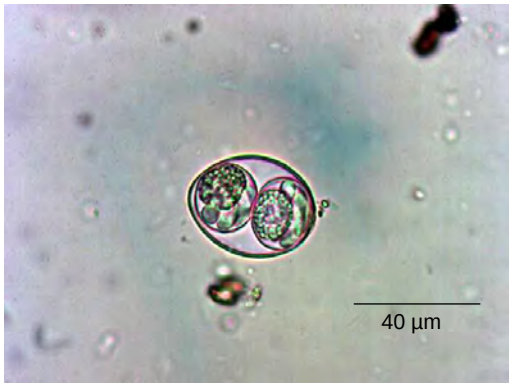

Figure 2 After incubation, oocysts of *Cystoisospora* spp. sporulate to contain two sporocysts, each with four sporozoites. (*Image credit: University of Melbourne parasite image library*)

Care should be taken to differentiate oocysts from those of *Eimeria* spp. or other coccidia (**Fig 3**) that may be mechanically ingested through coprophagy.

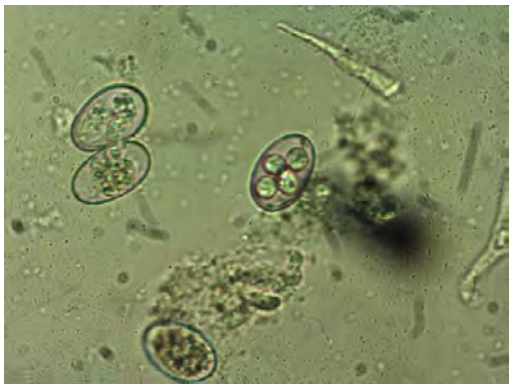

Figure 3 Following incubation, oocysts of *Eimeria* spp. sporulate to contain four sporocysts, each with two sporozoites. (*Image credit: University of Melbourne parasite image library*)

Treatment

Treat affected animals with oral sulfadimethoxine at 50 mg/kg daily for 5-20 days or oral trimethoprim-sulfonamide at 15-30 mg/kg for animals less than 4 kg and 30-60 mg/kg for animals more than 4 kg, for 6 days. Alternatively, a single dose of oral toltrazuril at 10 mg/kg or oral ponazuril at 50 mg/kg daily for 3 days can be used. If clinical signs persist, re-testing and re-treatment may be necessary.

Control

Pregnant females should be treated (as above) and bathed before whelping to remove sporulated oocysts on their hair coat. Ammonia-based disinfectants should be used for de-contamination of premises. For further control options refer to the **General Considerations and Recommendations** section.

Public health consideration

None.

Cryptosporidium (*Cryptosporidium canis*, *Cryptosporidium parvum*)

Cryptosporidium spp. are protozoa with a wide host-range. Transmission occurs by the faecal-oral route either directly or via contaminated food and water. Puppies are most susceptible to illness. *Cryptosporidium* is zoonotic.

Parasite: *Cryptosporidium canis*, *Cryptosporidium parvum*

Common name: Cryptosporidium

Host: Dogs, livestock, humans

Location of adults: Small intestine

Pre-patent period: 2-14 days

Distribution: Worldwide

Transmission route: Oral (ingestion of oocysts directly or via contaminated food and water)

Zoonotic: Yes

Distribution

Worldwide.

Clinical signs

Infection with *Cryptosporidium* is often asymptomatic, especially in adult dogs. If clinical disease manifests, it is usually associated with young and immunosuppressed animals. Cryptosporidiosis in dogs tends to manifest as an acute bout of water diarrhoea, which usually resolves in 7-10 days but may be chronic if the host is immunocompromised.

Diagnosis

Oocysts are challenging to identify (**Fig 1**). Specialized stains such as the Ziehl-Neelsen or modified acid-fast staining of direct faecal smears (**SOP 6**) reveal red or pink 5-6 µm oocysts (**Fig 2**). Commercial rapid immunodiagnostic coproantigen kits are useful in-house diagnosis. PCR-testing may be available through commercial laboratories.

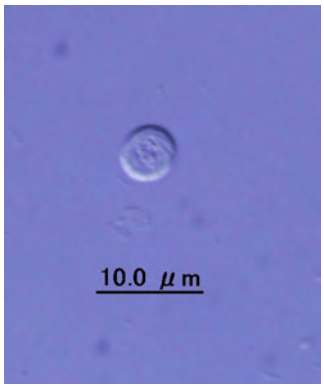

Figure 1 Unstained *Cryptosporidium* oocyst on a faecal float. (*Image credit: Dr. B. K. Linh*)

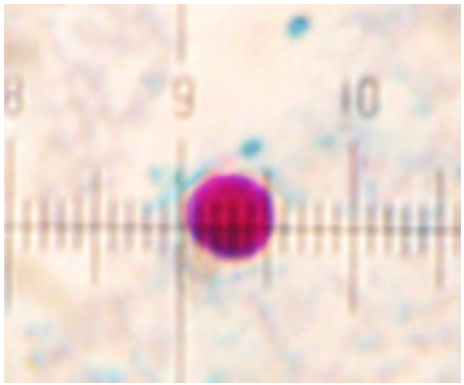

Figure 2 *Cryptosporidium* oocyst stained using modified acid-fast staining. (*Image credit: Dr. B. K. Linh*)

Treatment

A number of off-label drugs and regimes, for example, using azithromycin, paromomycin, tylosin and nitazoxanide, have been used with some success for the resolution of cryptosporidiosis-related diarrhoea, however, have not been supported with controlled studies. None of these regimes has proven to result in the elimination of oocyst excretion.

Control

For control options, refer to the **General Considerations and Recommendations** section.

Public health considerations

Zoonotic transmission of *C. parvum* may occur in healthy individuals, with the most common source being calves and other humans. Rare cases of infection with *C. canis* have been reported in children or patients with immunosuppressive disorders.

Parasites of Other Systems

Heartworm (*Dirofilaria immitis*)

Dirofilaria immitis (heartworm) is a filarial nematode of dogs (and cats) transmitted by mosquitoes. It is a leading cause of right-side congestive heart failure, pulmonary disease and death in dogs in the tropics and sub-tropics. It is zoonotic, although only rarely causes illness in people.

Parasite: *Dirofilaria immitis*

Common name: Heartworm

Host: Dogs, wild canids

Pre-patent period: 6-9 months

Location of adults: Pulmonary artery

Distribution: Tropical and sub-tropical regions

Transmission route: Mosquitoes

Zoonotic: Yes

Distribution

Worldwide, although occurrence in some cooler climatic regions can be limited. Widespread in tropical and sub-tropical regions.

Clinical signs

Clinical signs relate to progressive chronic heartworm disease. In early stages of infection, dogs are usually asymptomatic but they advance over a period of months-to-years to manifest chronic progressive pulmonary and congestive heart disease. At this stage, clinical signs may include cough, exercise intolerance, weight loss and lethargy. As the disease progresses, dyspnoea, tachypnoea, haemoptysis, tachycardia, cardiac murmur, syncope, hepatomegaly, ascites and renal insufficiency may ensue. Caval syndrome (**Fig 1**) with haemolysis may develop, creating additional signs of laboured breathing, pallor, icterus and haemoglobinuria.

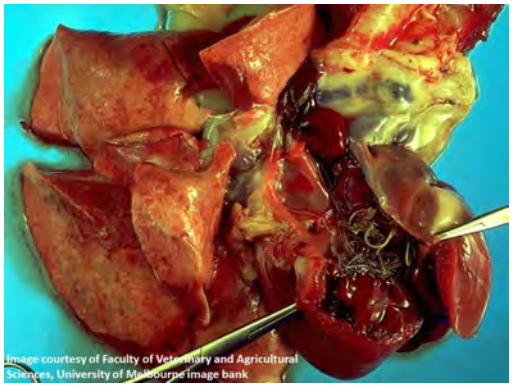

Figure 1 Adult heartworms recovered from a dog with caval syndrome. (*Image credit: The University of Melbourne, parasite image library*)

Diagnosis

Based on history (e.g. lack of heartworm prophylaxis, coughing) and physical examination findings, a diagnosis of heartworm disease should be confirmed using a commercial heartworm antigen detection test as well as a microfilarial detection test using a concentration technique; the modified Knott's or filtration test (**SOP 5)** for example. In many geographical locations circulating microfilarial densities peak in the late afternoon and evening, especially once the animal has eaten a meal. Blood collection during these periods will reduce the probability of a false negative microfilarial detection test. Care should be taken to morphologically differentiate (**Fig 2, Table 3**) microfilariae of *D. immitis* from other filarial parasites occurring in the area (e.g. *Dirofilaria repens*, *Acanthocheilonema* spp., *Brugia* spp.). Occult infections (lack of observed microfilariae) may complicate diagnosis.

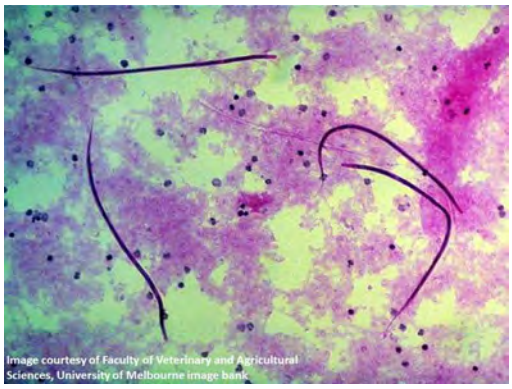

Figure 2 Microfilariae of *Dirofilaria immitis*. (*Image credit: The University of Melbourne parasite image library*)

Table 3 Summary of filarial species infecting dogs and their distinguishing features

Filarial species	Microfilarial morphology [1]		
	Special features (when fixed in 2% formalin)	Length (µm)	Width (µm)
Dirofilaria immitis	Unsheathed, tapered head, straight tail, cephalic space 6.07 ± 1.93 µm	295 - 308	6.0 – 6.6
Dirofilaria repens	Unsheathed, blunt head, ± curved tail ("umbrella handle"), cephalic space 2.92 ± 1.18 µm	358 - 380	8.0 – 9.4
Acanthocheilonema reconditum	Unsheathed, blunt head, curved tail ("umbrella handle")	254 - 271	4.0 – 5.3
Acanthocheilonema dracunculoides		252 – 266	5.0 -6
Acanthocheilonema sp. nov. ? (Ladakh, India)		130 – 180	4.8 - 6.0
Cercopithifilaria grassi		567	Not available
Microfilaria auquieri	Unsheathed	58 - 102	Not available

Microfilaria ochmanni	Sheathed	320	Not available
Brugia malayi	Sheathed, cephalic space: 6.3 – 6.7µm	254 - 234	5.99-7.99
Brugia pahangi	Sheathed, cephalic space: 6.4µm	200 - 189	4 - 5
Brugia ceylonensis	Sheathed, blunt tail, cephalic space: 6.3 – 6.7µm	220 – 275	Not available

Imaging tools e.g., radiography (**Fig 3**) and echocardiography may aid diagnosis and determine the severity of disease.

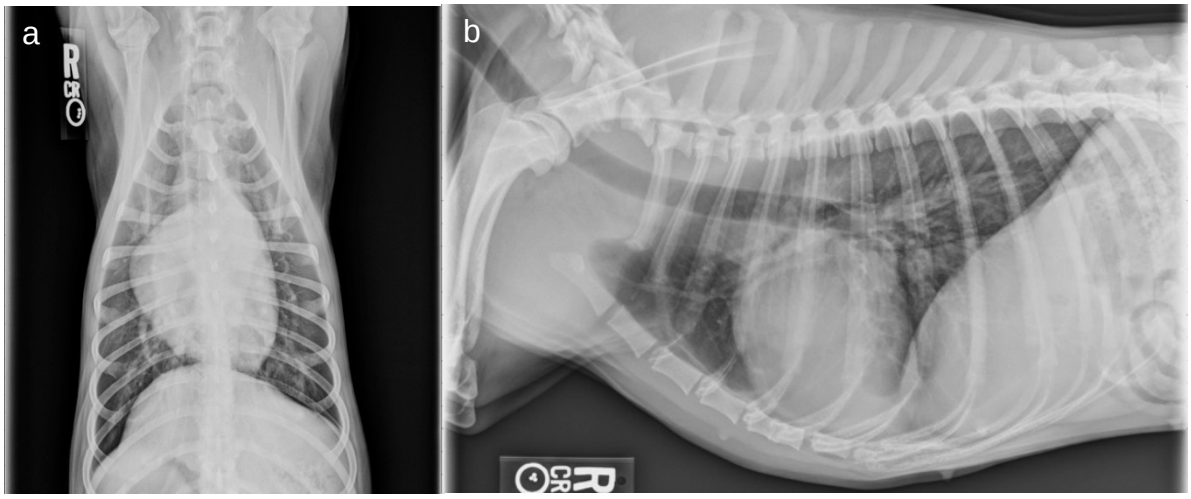

Figure 3 Ventrodorsal (a) and left lateral (b) thoracic radiographs of a dog with moderate heartworm disease. (*Image credit: Dr. A. Sharma and Ms. M. Savadelis*)

Treatment

Coughing dogs with confirmed heartworm infection should be managed symptomatically with anti-inflammatory doses of corticosteroid while specific treatment (see below) is started. Dogs exhibiting severe clinical signs of heartworm disease should be stabilized **before** administering an adulticide by administration of ancillary medications such as glucocorticosteroids, diuretics, vasodilators, positive inotropic agents, and fluid therapy.

The following guidelines are based on those developed and refined over decades by the American Heartworm Society (https://www.heartwormsociety.org).

Dogs should be exercise-restricted, commenced on monthly or injectable macrocyclic lactone and doxycycline (10 mg/kg twice daily, for 4 weeks) **two months before** the initial administration of melarsomine dihydrochloride. Melarsomine should be administered at 2.5 mg/kg by deep intramuscular injection into the epaxial lumbar muscles, and a second and third dose administered again after one month, 24 hours apart.

In countries where melarsomine is unavailable, a ‘slow-kill’ regime using a combination of a macrocyclic lactone and doxycycline may be the only adulticidal option.

Oral ivermectin 6 µg/kg administered at 2-weekly intervals for 6 months together with doxycycline 10 mg/kg twice daily for 30 days, resulted a negative heartworm antigen test in 72% of dogs tested 12 months following the commencement of therapy [2].

Alternatively, oral ivermectin 6µg/kg administered weekly; in combination with doxycycline 10 mg/kg twice daily, administered for 6 weeks, at monthly intervals for a total of 36 weeks, had an efficiency of 78% against adult heartworms [3].

Heartworm antigen testing should be performed after 6 months of commencing therapy and every 3 months thereafter. The dog is considered heartworm negative after two consecutive negative antigen tests. If the dog is still positive, doxycycline therapy should be repeated.

Veterinarians should be made aware that during the entire course of slow-kill therapy pathology may continue to develop while the adults are alive. Complications or sudden death due to pulmonary emboli owing to death of adult worms may also occur. Exercise restriction is recommended throughout this time.

TroCCAP strongly advocates the use of melarsomine as an adulticide. "Slow-kill" may promote the risk of heartworm developing resistance to macrocyclic lactones.

Control

Chemoprophylaxis with a macrocyclic lactone should commence as early as possible (6-8 weeks of age), according to labelled recommendations. Dogs should be tested for heartworm on an annual basis regardless of prophylaxis use to monitor product efficacy and owner compliance. Mosquito control through the use of repellents e.g. pyrethroids should be applied to the dog.

Public health considerations

Dirofilaria immitis may rarely infect humans. In humans, the worms may be found within granulomas in the lung that resemble 'coin-like' lesions on radiographs. Most reported human cases are asymptomatic, however in rare cases, cough, chest pain and haemoptysis may ensue. Ocular infections with adult worms have also been reported.

References

[1] Magnis J, Lorentz S, Guardone L, Grimm F, Magi M, Naucke T, Deplazes P. Morphometric analyses of canine blood microfilariae isolated by the Knott's test enables *Dirofilaria immitis* and *D. repens* species-specific and *Acanthocheilonema* (syn. *Dipetalonema*) genus-specific diagnosis. *Parasit Vectors.* 2013;6:48.

[2] Grandi G, Quintavalla C, Mavropoulou A, Genchi M, Gnudi G, Bertoni G, Kramer L. A combination of doxycycline and ivermectin is adulticidal in dogs with naturally acquired heartworm disease (*Dirofilaria immitis*). *Vet Parasitol*. 2010;169:347-351.

[3] Bazzocchi C, Mortarino M, Grandi G, Kramer LH, Genchi C, Bandi C, Genchi M, Sacchi L, McCall JW. Combined ivermectin and doxycycline treatment has microfilaricidal and adulticidal activity against *Dirofilaria immitis* in experimentally infected dogs. *Int J Parasitol*. 2008;38:1401-1410.

French Heartworm (*Angiostrongylus vasorum*)

Angiostrongylus vasorum is a strongylid nematode parasitizing the pulmonary arteries (**Fig 1**) of dogs and wild canids, responsible for often severe respiratory disorders but also other associated clinical signs. Infection of dogs is via ingestion of intermediate (gastropods) or paratenic (microvertebrates) hosts.

Parasite: *Angiostrongylus vasorum*

Common name: French heartworm

Host: Canids, rarely other carnivores

Pre-patent period: 6-8 weeks

Location of adults: Pulmonary arteries

Distribution: Europe, North America, South America

Transmission route: Oral [ingestion of intermediate (slugs, snails) or paratenic hosts (frogs, chicken)]

Zoonotic: No

Distribution

Angiostrongylus vasorum is distributed in Europe, North America and South America (Brazil, Argentina, Bolivia, and Colombia).

Clinical signs

Some cases are asymptomatic, some are symptomatic. Common clinical signs include respiratory disorders (cough, dyspnoea), lethargy, coagulopathy, neurological signs. Chronic cases present also anaemia, weight loss, fever, and weakening.

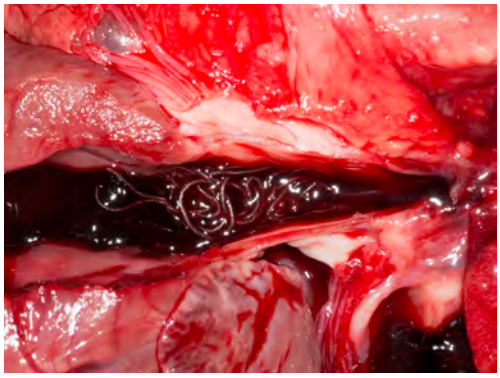

Figure 1 *Angiostrongylus vasorum* in the pulmonary arteries of a red fox. (*Image credit: Dr. A. D. Mihalca*)

Diagnosis

Various approaches are available for diagnosis. Detection of L1 in faeces (Baermann method) is possible during the patent phase of the infection (**Fig 2**). Differential diagnosis with other larval stages (i.e., *Crenosoma*, *Filaroides*) should be made. Various serologic methods are also available, including a rapid in-clinic test for the detection of circulating antigens.

Figure 2 First-stage larva of a canine lungworm containing a 'kink' in the tail. (*Image credit: Dr. R. Traub*)

Treatment

Macrocyclic lactones are effectively used for the treatment of canine angiostrongylosis. Moxidectin (2.5 mg/kg, spot on), repeated after 4 weeks or milbemycin oxime (0.5 mg/kg PO), weekly for 4 weeks. Another option is fenbendazole (25-50 mg/kg PO), daily for 3 weeks. To avoid thrombotic complications, supportive therapy is needed.

Control

Monthly chemoprophylaxis with or milbemycin oxime or moxidectin are recommended in endemic areas. If possible, access of dogs to gastropods or paratenic hosts should be prevented.

Public health considerations

None.

Subcutaneous Dirofilaria (*Dirofilaria repens*)

Dirofilaria repens is a filarial nematode of dogs (and cats) transmitted by mosquitoes. The adult worm commonly found in sub-cutaneous tissue deposit microfilariae that circulate in blood. *Dirofilaria repens* is zoonotic.

Parasite: *Dirofilaria repens**
Common name: Subcutaneous Dirofilaria
Host: Dogs, wild canids
Pre-patent period: 120-180 days
Location of adults: Subcutaneous tissue and peri-muscular fasciae
Distribution: Africa, southern and central Europe, Asia
Transmission route: Mosquitoes
Zoonotic: Yes

***** Other *Dirofilaria* spp. or strains have been reported as causative agents of subcutaneous dirofilariosis in dogs (e.g., "*Candidatus* Dirofilaria hongkongensis"), but further research is needed to confirm their identity and/or pathogenic role.

Distribution

Dirofilaria repens has been reported in Africa, the Middle East, Europe and Asia.

Clinical signs

Infection may be asymptomatic or most commonly present as generalised dermatological lesions as a result of a hypersensitivity reaction to microfilariae. This includes pruritus, erythema, papule formation and secondary alopecia and excoriations [1]. Subcutaneous nodules harbouring adult worms are occasionally observed.

Diagnosis

Identification of circulating microfilariae in whole blood using a microfilarial concentration technique (e.g. the modified Knott's method (**SOP 5**)) is the diagnostic test of choice. If a nodule is observable, cytological examination of the fine needle aspirate may reveal the presence of microfilaria. Currently, no serological test kits for the detection of *D. repens* are available. In many geographical locations circulating microfilarial densities peak in the late afternoon and evening, especially once the animal has eaten a meal. Blood collection during these periods will reduce the probability of a false negative microfilarial detection test. Care should also be taken to morphologically differentiate microfilariae of *D. repens* from other filarial parasites occurring in the area (see **Table 3**) (e.g. *D. immitis*, *Acanthocheilonema* spp., *Brugia* spp.). Occult infections (lack of observed microfilariae) may complicate diagnosis.

Treatment

Treatment is indicated in all positive cases to eliminate the dog as a source of infection to other animals as well as humans. No adulticide therapy for this parasite is registered. An off-label use of two doses of melarsomine hydrochloride at 2.5 mg/kg IM into the lumbar epaxial musculature, 24 hours apart, combined with a single sub-cutaneous injection of doramectin as a microfilaricidal treatment at 0.4 mg/kg 5 days after the initial adulticide therapy, was shown effective as an adulticidal and microfilaricidal therapy [2]. Alternatively, spot-on products containing moxidectin and selamectin are also efficacious as a microfilaricide and

when used for longer periods are also efficacious adulticides when administered at labelled monthly intervals [3,4]. Doxycycline 10 mg/kg PO daily for 30 days combined with a single dose of ivermectin 6 µg/kg PO every 15 days for 6 months is also reported as an effective microfilaricide [5]. When present, surgical removal of nodules may be warranted.

Control

Macrocyclic lactones given at labelled recommendations for the prevention of heartworm are also effective for the prevention of *D. repens*. In endemic areas, chemoprophylaxis with a macrocyclic lactone should commence as early as possible (6 - 8 weeks of age), according to labelled recommendations. Mosquito control through the use of repellents (e.g. pyrethroids) should be applied to the dog.

Table 3 Summary of filarial species infecting dogs and their distinguishing features

Filarial species	Microfilarial morphology [1]		
	Special features (when fixed in 2% formalin)	Length (µm)	Width (µm)
Dirofilaria immitis	Unsheathed, tapered head, straight tail, cephalic space 6.07 ± 1.93 µm	295 - 308	6.0 – 6.6
Dirofilaria repens	Unsheathed, blunt head, ± curved tail ("umbrella handle"), cephalic space 2.92 ± 1.18 µm	358 - 380	8.0 – 9.4
Acanthocheilonema reconditum	Unsheathed, blunt head, curved tail ("umbrella handle")	254 - 271	4.0 – 5.3
Acanthocheilonema dracunculoides		252 – 266	5.0 -6
Acanthocheilonema sp. nov. ? (Ladakh, India)		130 – 180	4.8 - 6.0
Cercopithifilaria grassi		567	Not available
Microfilaria auquieri	Unsheathed	58 - 102	Not available
Microfilaria ochmanni	Sheathed	320	Not available
Brugia malayi	Sheathed, cephalic space: 6.3 – 6.7µm	254 - 234	5.99-7.99
Brugia pahangi	Sheathed, cephalic space: 6.4µm	200 - 189	4 - 5
Brugia ceylonensis	Sheathed, blunt tail, cephalic space: 6.3 – 6.7µm	220 – 275	Not available

Public health considerations

Dogs act as reservoirs for human infection. In humans, the worms undergo migration through the tissues and may be found within nodular lesions under the skin, eyelids and

periorbital tissue, mouth, female breasts and male genitals. These nodules are often confused with neoplasms and eventually may be removed surgically.

References

[1] Talerro W. Clinical aspects of dermatitis associated with *Dirofilaria repens* in pets: A review of 100 canine and 31 feline cases (1990–2010) and a report of a new clinic case imported from Italy to Dubai. *J Parasitol Res*. 2011;578385.

[2] Baneth G, Volansky Z, Anug Y, Favia G, Bain O, Goldstein RE, Harrus S. *Dirofilaria repens* infection in a dog: diagnosis and treatment with melarsomine and doramectin. *Vet Parasitol*. 2002;105:173-178.

[3] Petry G1, Genchi M, Schmidt H, Schaper R, Lawrenz B, Genchi C. Evaluation of the adulticidal efficacy of imidacloprid 10 %/moxidectin 2.5 % (w/v) spot-on (Advocate®, Advantage® Multi) against *Dirofilaria repens* in experimentally infected dogs. *Parasitol Res.* 2015;114 Suppl 1:S131-44.

[4] Jacsó O, Fok E, Kiss G, Kökény G, Lang Z: Preliminary findings on the efficacy of selamectin in the treatment of dogs naturally infected with *Dirofilaria repens*. *Acta Vet Hung.* 2010;58:405-412.

[5] Giannelli A, Ramos RA, Traversa D, Brianti E, Annoscia G, Bastelli F, Dantas-Torres F, Otranto D. Treatment of *Dirofilaria repens* microfilariaemia with a combination of doxycycline hyclate and ivermectin. *Vet Parasitol*. 2013;197:702-704.

Oriental Eyeworm (*Thelazia callipaeda*)

Thelazia callipaeda is a spirurid of dogs, which can also be found in cats and wildlife such as foxes and hares. This parasite is transmitted by secretophagous flies, which is a fruit fly that feeds on lachrymal secretions of mammals. It is zoonotic.

Parasite: *Thelazia callipaeda*

Common name: Oriental eyeworm

Hosts: Dogs, cats, several wildlife species, humans

Pre-patent period: 3 weeks

Location of adults: Conjunctival sac

Distribution: Some parts of Asia and Europe

Transmission route: Secretophagous flies (*Phortica variegata*, *Phortica okadai*)

Zoonotic: Yes

Distribution

Thelazia callipaeda has been reported in several parts of Europe and Asia, including China, India, Bangladesh, Myanmar, Indonesia, Japan, Korea, Taiwan, and Thailand.

Clinical signs

In most cases, *T. callipaeda* infection in dogs is asymptomatic, but clinical signs may include mild conjunctivitis, blepharitis, epiphora, periocular pruritus and, in severe cases, corneal oedema and keratitis (**Fig 1**). Blindness may eventually occur in severe cases that are left untreated.

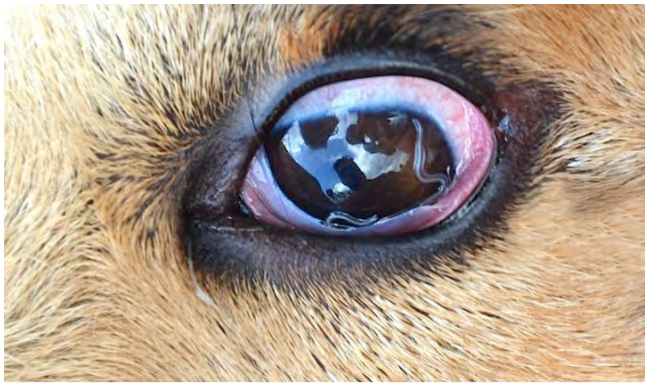

Figure 1 *Thelazia callipaeda* in the eye of a dog.
(*Image credit: Dr. D. Otranto and Dr. F. Dantas-Torres, DOI: 10.1186/s13071-015-0881-7*)

Diagnosis

Diagnosis is achieved by visual inspection and retrieval of adult worms in the eye of infected hosts. First-stage larvae of the parasite may also be found in ocular secretions.

Treatment

Mechanical removal of the worms by flushing saline solution in the affected eye is usually successful. Other treatments involve off-label use of anthelmintics. A single application of topical imidacloprid plus moxidectin (2.5 mg/kg) killed worms within 7 days of application.

Two oral doses of milbemycin oxime (0.5 mg/kg) administered one week apart reached 100% efficacy 28 days following treatment. Alternatively, a single dose of 200 µg/kg oral ivermectin achieved 100% efficacy in 25 days.

Control

Control of *T. callipaeda* infections in dogs may be achieved by avoiding wooded environments inhabited by *Phortica variegata* and by treating infected animals.

Public health considerations

Several cases of human thelaziosis have been recorded in Asia and Europe, especially in people living near wooded environments, where the natural life cycle of this parasite takes place. Clinical signs resemble those of dogs listed above.

Onchocerca (*Onchocerca lupi*)

Onchocerca lupi is a spirurid helminth of dogs, which also infects cats and wolves. Biting midges are suspected vectors, but a definitive proof of their vector competence is currently lacking. It is zoonotic.

Parasite: *Onchocerca lupi*

Common name: Onchocerca

Hosts: Dogs, wolf, cats, humans

Pre-patent period: Unknown

Location of adults: Subconjunctiva and retrobulbar space

Distribution: United States, Europe, Asia and Africa

Transmission route: Unknown vectors (black flies?)[1]

Zoonotic: Yes

Distribution

Onchocerca lupi has been reported in subtropical regions including the southern United States, Greece, Portugal, Romania, Turkey, Tunisia, and Iran.

Clinical signs

Most *O. lupi*-infected dogs remain asymptomatic, showing no apparent clinical signs. Some dogs may present ocular lesions, including ocular nodules that are often evident on the eyelids, conjunctiva, and sclera (**Fig 1**).

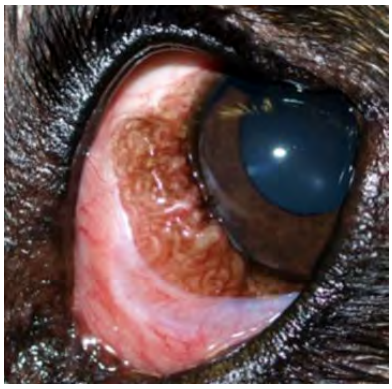

Figure 1 Subconjunctival masses containing *Onchocerca lupi.* (*Image credit: Dr. D. Otranto and coworkers, DOI: 10.1186/s13071-015-0699-3*)

Diagnosis

The diagnosis of *O. lupi* infection in dogs is based on the detection of characteristic microfilaria in skin snips (**Fig 2**) and/or on the identification of adult worms recovered from ocular nodules. Imaging tools (e.g., ultrasound scan, computed tomography and magnetic resonance imaging) may be used for detecting the presence of adult worms in anatomical regions that cannot be easily accessed during routine ophthalmologic examination.

Figure 2 *Onchocerca lupi* microfilaria. (*Image credit: Dr. R. P. Lia*)

Treatment

The only effective treatment for canine onchocercosis demonstrated so far is the surgical removal of adult worms from accessible nodules (**Fig 3**).

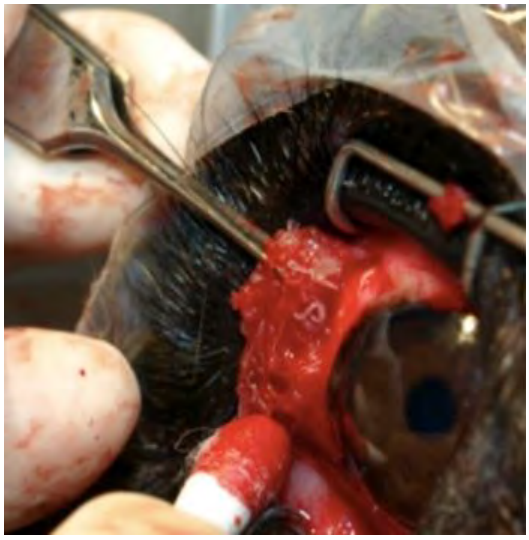

Figure 3. Surgical removal of a subconjunctival mass containing *Onchocerca lupi.* (*Image credit: Dr. D. Otranto and coworkers, DOI: 10.1186/s13071-015-0699-3*)

Control

As the mode of transmission of this enigmatic parasite remains unknown, no effective control measure has yet been proposed.

Public health considerations

After the first evidence of human infection by *O. lupi* in Turkey, new human cases have been described in Tunisia, Germany, Hungary, Greece, Portugal, Iran and the United States. Human patients usually present painless subconjunctival nodules which require surgical intervention. Interestingly, American patients have not had subconjunctival nodules but spinal, orbital, and sub-dermal nodules.

References

[1] Hassan HK, Bolcen S, Kubofcik J, Nutman TB, Eberhard ML, Middleton K, Wekesa JW, Ruedas G, Nelson KJ, Dubielzig R, De Lombaert M, Silverman B, Schorling JJ, Adler PH,

Unnasch TR, Beeler ES. Isolation of *Onchocerca lupi* in dogs and black Flies, California, USA. *Emerg Infect Dis*. 2015;21:789-796.

Lymphatic Filarial Worms (*Brugia malayi, Brugia pahangi*)

Brugia malayi and *Brugia pahangi* are nematodes that cause lymphatic filariasis in humans. Dogs are suspected to be reservoirs of human infection and rarely show clinical signs when infected.

Parasite: *Brugia malayi, Brugia pahangi*
Common name: Lymphatic filarial worms
Hosts: Humans, dogs, cats
Location in host: Free in bloodstream
Distribution: Indonesia, Malaysia, Thailand, India
Transmission route: Mosquitoes
Zoonotic: Yes

Distribution

Brugia malayi and *B. pahangi* are restricted to Southeast Asia and India.

Clinical signs

Dogs infected with *B. malayi* and *B. pahangi* are a rare occurrence and mostly remain asymptomatic. There have been limited reports of infected dogs developing lymphadenopathy and lymphedema. Studies have shown that genetically inherited traits determine the clinical outcome of infection in dogs.

Diagnosis

The diagnosis of *Brugia* spp. infection can be made upon detection of the microfilariae in wet blood mounts and thin blood smears via light microscopy. Serological assays such as ELISA can also be used to confirm a diagnosis through the detection of antibodies or antigen. PCR with sequencing are useful for detection of low parasitaemia and for species determination.

Treatment

Brugia infection in dogs can be treated with moxidectin, selamectin, doramectin and ivermectin.

Control

Minimizing dog contact with vectors by using topical repellents and insecticides such as collars and spot-on formulations (e.g. permethrin, flumethrin, deltamethrin).

Public health considerations

Brugia malayi and *B. pahangi* are both zoonotic and there have been several reports in humans in endemic areas.

Liver Flukes (*Opisthorchis viverrini*, *Clonorchis sinensis*)

Opisthorchis viverrini and *Clonorchis sinensis* are trematodes of fish-eating mammals including dogs, cats and humans in Asia. Liver flukes are zoonotic.

Parasite: *Opisthorchis viverrini*, *Clonorchis sinensis*

Common name: Southeast Asian liver fluke, Chinese or Oriental liver fluke

Hosts: Fish-eating mammals such as dogs, cats, pigs, and humans

Pre-patent period: 3-4 weeks

Location of adults: Bile duct, liver, gallbladder, pancreatic duct

Distribution: Southeast Asia and Far East Asia

Transmission route: Eating raw or undercooked freshwater fish infected with metacercariae

Zoonotic: Yes

Distribution

Opisthorchis viverrini has been reported in Thailand, Laos, central Vietnam and Cambodia, whereas *C. sinensis* has been reported in Korea, China, Taiwan and northern Vietnam.

Clinical signs

In most cases, liver fluke infection in dogs is asymptomatic. When clinical signs occur they include lethargy, diarrhoea and dehydration. Migration of immature flukes can cause acute hepatitis and pancreatitis.

Diagnosis

The diagnosis of liver fluke infection in dogs is based on the detection of characteristic operculated eggs with a fully developed miracidium (**Fig 1**) by faecal sedimentation (**SOP 4**).

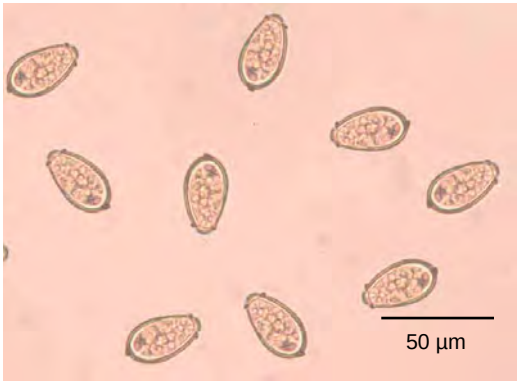

Figure 1 Liver fluke eggs with distinct 'shoulder' below the operculum ('cap'). (*Image credit: Shutterstock*)

Treatment

Off-label use of praziquantel 40 mg/kg given as a single oral dose is reported effective at killing adult liver flukes.

Control

Owners should be advised not to feed their dog raw or undercooked freshwater fish. For further control options, refer to the **General Considerations and Recommendations** section.

Public health considerations

Humans become infected through the ingestion of undercooked fish infected with metacercariae of liver flukes. Dogs may act as reservoirs with eggs excreted contaminating the environment and ultimately enabling infection in the fish. Humans infected with liver fluke are mostly asymptomatic, but chronic infection may lead to biliary and hepatic disease and cholangiocarcinoma.

Lung Flukes (*Paragonimus* spp.)

There are numerous species of *Paragonimus* known to infect dogs through the consumption of undercooked crustaceans. These trematodes are capable of causing serious clinical signs and may be fatal if left untreated. Many lung fluke species are zoonotic.

Parasite: *Paragonimus westermani*, *Paragonimus heterotremus*, *Paragonimus skrjabini* complex, *Paragonimus mexicanus*, among others (at least 28 species)

Common name: Lung flukes

Host: Humans, canids, felids, rodents

Pre-patent period: 60-90 days

Location of adults: Lung parenchyma

Distribution: East Asia, Central and South America, Africa

Transmission route: Oral (ingestion of crustaceans or wild boar)

Zoonotic: Yes

Distribution

Paragonimus spp. are distributed throughout the tropics. *Paragonimus westermani*, *P. skrjabini* complex and *P. heterotremus* are distributed through India and SE Asia. *Paragonimus mexicanus*, *P. peruvianus*, *P. ecuadoriensis* and *P. inca* in Central and South America. Not all species of lung flukes in Central and South America are reported to infect dogs, however, infection is possible if access to infected hosts is present.

Clinical signs

Infection may be asymptomatic or include fever, cough, haemoptysis and dyspnoea. Sudden death owing to bilateral pneumothorax has also been reported. Ectopic infections may produce subcutaneous nodule formation, lymphadenopathy, lymphadenitis and cellulitis.

Diagnosis

The diagnosis of lung fluke infection in dogs is based on the detection of characteristic large, oval, tanned operculated eggs with a fully developed miracidium (**Fig 1**) by faecal sedimentation (**SOP 4**).

Figure 1 *Paragonimus* egg with a distinct operculum ('cap'). (*Image credit: Shutterstock*)

Thoracic radiographs may reveal pulmonary nodules, congestion, pleural effusion and pneumothorax.

Treatment

Off-label use of oral praziquantel given at 75 mg/kg/day (can be divided) for 2 days is reported effective at killing adult lung flukes.

Control

Owners should be advised not to feed their dog raw or undercooked crustaceans (e.g. crabs, crayfish, prawns) or wild boar/pig meat. For further control options, refer to the **General Considerations and Recommendations** section.

Public health considerations

Humans become infected through the ingestion of undercooked crustaceans or pork infected with metacercariae of lung flukes. Dogs may act as reservoirs for human infection by contaminating the environment with lung fluke eggs. Humans infected with lung flukes may present with cough, often with haemoptysis. Ectopic infections are also possible.

Tongue Worm (*Linguatula serrata*)

Linguatula serrata is a nasal pentastomid parasite of dogs and wild canids worldwide, responsible for mild to severe rhinitis and it is transmitted by ingestion of organs of infected intermediate hosts (primarily herbivores).

Parasite: *Linguatula serrata*

Common name: Tongue worm

Host: Canids (**Fig 1**), rarely felids and humans

Pre-patent period: 6 months

Location of adults: Nasal cavities

Distribution: Worldwide, mostly Middle East (Iran)

Transmission route: Oral [ingestion of organs (lymph nodes) of intermediate hosts (ruminants, camels, rodents, lagomorphs)] (**Fig 2**)

Zoonotic: Yes

Distribution

Linguatula serrata is distributed worldwide, with higher prevalence in Iran, Lebanon, India, and some parts of Africa (i.e., Nigeria), related mainly to the traditional pastoralist life style. In other parts of the world, the occurrence is sporadic.

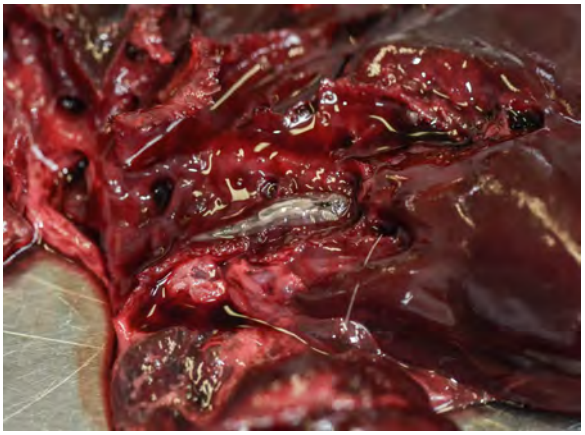

Figure 1 An adult female of *Linguatula serrata* in the nasal cavity of a red fox. (*Image credit: Dr. A. D. Mihalca*)

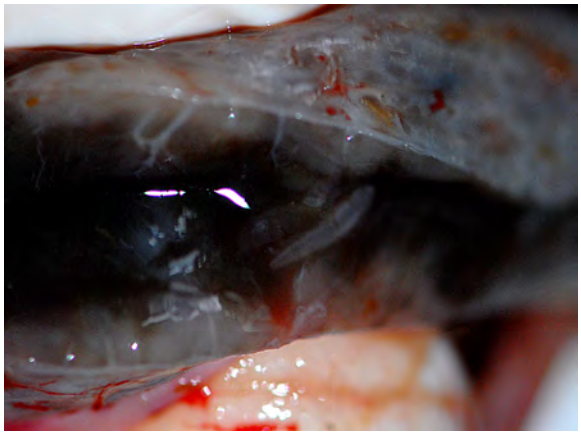

Figure 2 Larva of *Linguatula serrata* in the lymph node of a goat. (*Image credit: Dr. A. D. Mihalca*)

Clinical signs

Infected dogs show mild to severe clinical signs of rhinitis (unilateral or bilateral), with sneezing, nosebleed/epistaxis, and evident signs of nasal foreign body (pawing at the nose). Severity of clinical signs depends on the intensity of infection.

Diagnosis

Eggs (90 x 70 µm, larvated) can be detected in faeces by standard flotation (**SOP 1**) or in the nasal discharge, by fresh direct microscopic examination. However, egg shedding is intermittent and can be absent if infection is mono-sexual. Differential diagnosis should be made with other nasal foreign body syndromes.

Treatment

There is no approved drug for the treatment of canine nasal linguatulosis. Nasal flushing using warm salty water can help detachment of parasites. Surgical removal is unfeasible, as parasites may be attached deep in the sinuses. Macrocyclic lactones (milbemycin oxime, ivermectin) have been used off-label.

Control

No chemoprophylaxis is available. The access of dogs to raw organs and slaughterhouse offal should be strictly controlled. For further control options, refer to the **General Considerations and Recommendations** section.

Public health considerations

Humans can act both as accidental definitive host, following consumption of raw organs with nymphs, when a severe nasopharyngeal linguatulosis and/or severe allergic reactions develop (known as "*halzoun*"). Accidental ingestion of eggs from dog faeces, results is asymptomatic visceral linguatulosis.

Giant Kidney Worm (*Dioctophyme renale*)

The giant kidney worm is a parasitic nematode that locates in the right kidney, which may result in destruction of the functional tissue or within the peritoneal cavity.

Parasite: *Dioctophyme renale*

Common name: Giant kidney worm

Host: Dogs, mustelids, cats, humans

Pre-patent period: 2 to 6 months

Location of adults: Right kidney, peritoneal cavity

Distribution: Worldwide

Transmission route: Oral [ingestion of intermediate (oligochaete annelid; "aquatic worms") or paratenic hosts (fish or frogs)]

Zoonotic: Yes

Distribution

Dioctophyme renale is found worldwide, except in Africa and Oceania.

Clinical signs

Many dogs are asymptomatic due to compensatory hypertrophy of the unaffected kidney. Clinical signs may include haematuria, right kidney pain, right limb claudication, lumbar and abdominal pain and potentially paresis of the hind quarters. If the left kidney is also compromised, the dog will develop clinical signs of renal failure such as polydipsia and polyuria. Worms migrating within in the peritoneal cavity may produce abdominal pain.

Diagnosis

Eggs are passed via the ureteral lumen into the urine if there is at least one female worm present in the kidney. Brownish, thick-shelled eggs with bipolar pigs (68 x 45 µm) containing a single cell, can be detected by the examination of urine sediment under light microscopy. If the slide is covered with red cells they should be removed by means of acetic acid to enable visualisation of the eggs. Ultrasound can be used to visualise the worms within the kidney or peritoneum. *Dioctophyme* worms are red, the female can reach 1 m in length and 1 cm in diameter.

Treatment

Surgical removal of the worm is the only current treatment option.

Control

Do not allow dogs to drink from fresh-water bodies or ingest aquatic worms or frogs. Dogs should not be fed raw fish.

Public health considerations

Humans get infected in the same way as dogs, most commonly by ingesting undercooked fish and frog.

Babesia (*Babesia* spp.)

Babesia spp. are tick-transmitted piroplasms that infect erythrocytes and constitute one of the most common and significant diseases to affect dogs living in the tropics. Canine babesiosis in the tropics and subtropics is caused primarily by two species, *Babesia vogeli* ("large" form) and *Babesia gibsoni* ("small" form).

Parasite: *B. vogeli*, *B. gibsoni*, *Babesia rossi*, *Babesia vulpes*

Common name: Babesia

Host: Dogs and wild canids

Incubation period: 1-6 weeks

Location in host: Intraerythrocytic

Distribution: Worldwide

Transmission route: Ticks, transplacental, blood transfusion, fighting (*B. gibsoni*)

Zoonotic: No

Distribution

Babesia vogeli has a worldwide distribution, which may be partly explained by its association with the brown dog tick (*Rhipicephalus sanguineus sensu lato*). *Babesia gibsoni* has also been found worldwide, but its distribution in some countries (e.g., Brazil) is much more restricted as compared with *B. vogeli*. *Babesia rossi* is confined to sub-Saharan Africa. *Babesia vulpes* is mainly found in Europe and Asia, but in North America (Mississippi, US).

Clinical signs

In general, *Babesia gibsoni* is more pathogenic than *B. vogeli*, although the latter is an important cause of mortality in pups less than 12 weeks old. Pathogenicity is greatly influenced by concurrent infection, especially other diseases that cause anaemia (e.g. hookworm infection). Dogs that survive the initial infection become life-long carriers of the parasite despite appropriate treatment and resolution of the original signs. Recrudescence of intraerythrocytic parasites into the bloodstream and the redevelopment of clinical illness may occur at any time in these dogs following stressful situations, immunosuppressive therapy or concurrent disease.

Per-acute babesiosis is characterised by the rapid onset of collapse owing to hypotensive shock. Pale mucous membranes, rapid heart rate, weak pulse, profound weakness, mental depression, vomiting and seizures (occasionally) may be present. Fever may be present but hypothermia is a more consistent finding.

Dogs with acute babesiosis may have been unwell for a few days with non-specific signs such as anorexia, depression, vomiting and lethargy. Clinical findings include pale mucous membranes, dehydration, icterus and hepatosplenomegaly, petechiae and ecchymosis, red, brown or yellow-orange urine (haemoglobinuria), vomiting and diarrhoea.

Chronic babesiosis has also been associated with non-specific signs such as anorexia, weight loss, lymphadenopathy, nasal discharge, bleeding tendencies. It is possible that such cases have concurrent ehrlichiosis or other significant disease, and the signs are unlikely to be caused by babesiosis alone.

Diagnosis

A tentative diagnosis can be made in animals with a history of exposure to ticks and associated clinical signs. The aims of the diagnostic investigation for babesiosis should be to **i)** identify the *Babesia* parasite(s); **ii)** search for other infectious agents (especially *Ehrlichia* spp.); **iii)** assess the severity of the anaemia; and **iv)** assess the patient's overall health status (especially in per-acute cases). Identification of large and small *Babesia* parasites is made by microscopic examination of a stained peripheral or capillary blood smear (See **Figs 1 and 2**). Whole blood may also be subjected to PCR, where commercially available. Serological tests may detect antibodies to either or both *B. gibsoni* or *B. vogeli,* depending on their specificity. Serological tests may return false negative results in per-acute or acute primary infection.

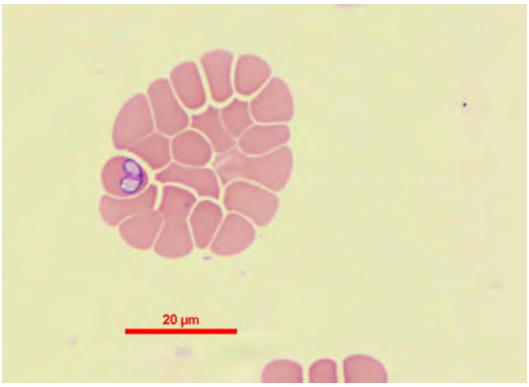

Figure 1 *Babesia vogeli* within a red blood cell. (*Image credit: Dr. P. Irwin*)

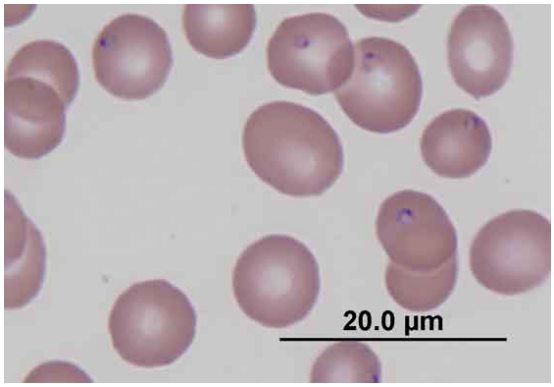

Figure 2 *Babesia gibsoni* within red blood cells. (*Image credit: Dr. P. Irwin*)

Treatment

For treatment options refer to **Table 2**.

Many drugs have been used to treat babesiosis, yet very few are consistently reliable. Few, if any, sterilize the infection, and most affected individuals harbour parasites after the treatment is finished. It should be noted that only a few drugs are efficacious against both forms of *Babesia*.

Blood transfusion in severely anaemic or careful administration of fluids in dehydrated animals may be indicated. Doxycycline at 10mg/kg/day PO (single or divided doses) for 21 days may be used if concurrent ehrlichiosis or other rickettsial diseases are suspected. Glucocorticoids (dexamethasone 0.2 mg/kg IV/SC or prednisolone 1-2 mg/kg/day divided doses for 5-10 days) have been recommended to ameliorate the immune-mediated haemolysis but the benefit in babesiosis is currently unproven.

The prognosis is variable and difficult to predict in tropical countries. This is probably more a reflection of the effects of concurrent diseases than the *Babesia* infection. As stated earlier, most dogs become lifelong carriers of *Babesia* parasites.

Table 2 Dose and efficacy of drugs used to treat babesiosis in dogs.

Host	Morphology	Drug	Recommended Dose and Frequency	Notes/Comments
Dog	Large (*B. vogeli*)	Imidocarb (dipropionate and dihydrochloride)	5-7 mg/kg SC or IM, repeat in 14 days	Pain at site of injection and nodule may develop at site of injection. Cholinergic signs (vomiting, diarrhoea) controlled with atropine (0.05 mg/kg SC)
	Large and Small	Phenamidine (isethionate)	15 mg/kg SC, once or repeat 24h	Nausea, vomiting and CNS signs are common side-effects
		Pentamidine (isethionate)	16.5 mg/kg IM, repeat 24h	Nausea, vomiting and CNS signs are common side-effects
		Diminazen aceturate	3.5 mg/kg IM, once	Unpredictable and idiosyncratic toxicity; CNS signs may be severe. Some preparations contain antipyrone
	Small (*B. gibsoni, B. vulpis*)	Parvaquone	20 mg/kg SC, once	
		Atovaquone plus azithromycin combination	13.3 mg/kg PO q8h for 10 days (atovaquone), 10 mg/kg q24h for 10 days (azithromycin)	Absorption of atovaquone is improved if given with food. Safe. Resistance reported.
		Clindamycin	25 mg/kg q12h PO	Causes morphological changes to piroplasms, efficacy uncertain
		Clindamycin, metronidazole plus doxycycline combination	25 mg/kg q12h PO (clindamycin), 15 mg/kg PO q12h (metronidazole), 5 mg/kg PO q12h (doxycycline)	
		Buparvaquone plus azithromycin combination	5 mg/kg IM, 2 d apart (buparvaquone) 10 mg/kg PO q 24 h, 10 d (azithromycin)	Absorption of atovaquone is improved if given with food.

Control

Prevent or reduce exposure to the tick vector by utilisation of registered long-acting acaricides (spot-on/collars) with continuous repel and kill activities (e.g. permethrin, flumethrin, deltamethrin, amitraz), according to labelled instructions. Blood donors should be screened and found free of vector-borne diseases, including *Babesia* spp. *Babesia* positive dams should not be bred and dog-fighting disallowed. For further information, refer to tick-control guidelines.

Public health considerations

None.

References

Checa R, Montoya A, Ortega N, González-Fraga JL, Bartolomé A, Gálvez R, Marino V, Miró G. Efficacy, safety and tolerance of imidocarb dipropionate versus atovaquone or buparvaquone plus

azithromycin used to treat sick dogs naturally infected with the *Babesia microti*-like piroplasm. *Parasit Vectors.* 2017;10:145.

Rangelia (*Rangelia vitalii*)

Rangelia vitalii is a tick-borne haemoprotozoan which affects domestic and wild canids from South America and can be detected in neutrophils, monocytes, endothelial cells and erythrocytes as well as free in the plasma.

Parasite: *Rangelia vitalii*

Common name: Rangelia

Hosts: Dogs and wild canids

Prepatent period: 5-15 days

Location: Intracellular (neutrophils, erythrocytes, endothelial cells and monocytes) and free in blood

Distribution: Brazil, Uruguay, Paraguay and Argentina

Transmission route: Ticks

Zoonotic: No

Distribution

Rangelia vitalii is spread in southern and south-eastern regions of Brazil, Uruguay, Argentina and recently, in Paraguay[1]. It is transmitted by the tick *Amblyomma aureolatum*[2].

Clinical signs

Dogs infected with *R. vitalii* may present intermittent fever, apathy, weight loss, hepatomegaly, splenomegaly, jaundice, generalized lymphadenopathy, hindlimb oedema, mucosal petechiae, haematemesis, and bloody diarrhoea. Typical clinical signs of canine rangeliosis include persistent bleeding from the nose (epistaxis), oral cavity, eyes and margins and lateral surface of the pinnae[3]. The latter is considered a characteristic clinical sign of this disease.

Diagnosis

The diagnosis of rangeliosis is based on the history, clinical signs, haemogram, peripheral blood smear evaluation and response to therapy. Parasitic stages may be found in erythrocytes, monocytes, neutrophils or free in peripheral blood smears. PCR assays are also available for detecting *R. vitalii* DNA in blood samples[3,4].

Treatment

The treatment of rangeliosis consists of off-label use of imidocarb dipropionate (6 mg/kg, IM). A second injection must be administered 15 days later[3]. To avoid the cholinergic effects, it is important to administer atropine (0.05 mg/kg, SC) 30 minutes before the imidocarb dipropionate.

Control

Infection with *R. vitalii* should be prevented and controlled by using long-lasting acaricides with repellent activity against ticks (e.g., permethrin, flumethrin, and deltamethrin), in accordance with manufacturer's instructions.

Public health considerations

None.

References

[1] Inácio EL, Pérez-Macchi S, Alabi A, Bittencourt P, Müller A. Prevalence and molecular characterization of piroplasmids in domestic dogs from Paraguay. *Ticks Tick Borne Dis*. 2019;10:321-327.

[2] Soares JF, Costa FB, Girotto-Soares A, Da Silva AS, França RT, Taniwaki SA, Dall'Agnol B, Reck J, Hagiwara MK, Labruna MB. Evaluation of the vector competence of six ixodid tick species for *Rangelia vitalii* (Apicomplexa, Piroplasmorida), the agent of canine rangeliosis. *Ticks Tick Borne Dis*. 2018;9:1221-1234.

[3] França RT, Da Silva AS, Loretti AP, Mazzanti CM, Lopes ST. Canine rangeliosis due to *Rangelia vitalii*: from first report in Brazil in 1910 to current day - a review. *Ticks Tick Borne Dis*. 2014;5:466-474.

[4] Soares JF, Girotto A, Brandão PE, França, RT, Da Silva AS, Lopes STA, Labruna M. Detection and molecular characterization of a canine piroplasm from Brazil. *Vet Parasitol*. 2011;180:203-208.

Hepatozoon (*Hepatozoon canis*)

Hepatozoonosis is a tick-borne apicomplexan protozoan distributed throughout the tropics and subtropics. Mild to severe disease may manifest in dogs.

Parasite: *Hepatozoon canis*

Common name: Hepatozoon

Hosts: Dogs and wild canids

Location in host: Gamonts in cytoplasm of neutrophils and monocytes

Distribution: Tropics and subtropics, worldwide

Transmission route: Oral (ingestion of infected ticks) (**Fig 1**), transplacental transmission

Zoonotic: No

Distribution

Hepatozoon canis in found in southern Europe, Africa, Asia, Latin America and parts of the USA, whereas *H. americanum* is restricted to south-eastern USA.

Clinical signs

Hepatozoon canis infects the haemolymphatic tissues and causes anaemia and lethargy. *Hepatozoon canis* infection varies from being subclinical in apparently healthy dogs to severe with lethargy, fever, cachexia and pale mucous membranes due to anaemia.

Figure 1 The brown dog tick, *Rhipicephalus sanguineus sensu lato*, a vector of *H. canis* (*Image credit: CDC/James Gathany; William Nicholson*)

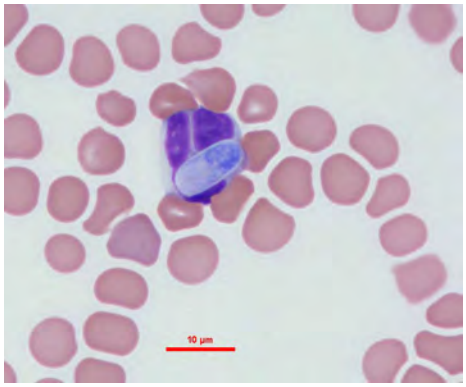

Figure 2 *Hepatozoon canis* gamont in a neutrophil of a stained capillary blood smear. (*Image credit: Dr. K. Kamyingkerd*)

Diagnosis

Hepatozoon canis infection is frequently diagnosed by microscopic detection of intracellular *H. canis* gamonts in neutrophils and monocytes in stained capillary blood smears (**Fig 2**). The degree of parasitaemia is directly proportional to the severity of clinical signs. PCR of whole blood for *H. canis* detection is sensitive and specific.

Treatment

Hepatozoon canis infection is treated with imidocarb dipropionate at 5-6 mg/kg IM or SC every 14 days until gamonts are no longer present in blood smears. The decrease of parasitemia is slow and usually requires several repeated imidocarb treatments.

Control

Prevention consists of the use of topical acaricides and environmental parasiticides. Furthermore, it is recommended to avoid the dog ingesting ticks while scavenging or grooming.

Public health considerations

Hepatozoon canis is not zoonotic. *Hepatozoon* infection in humans has not been described except for a single case in which the species was not identified.

Leishmania (*Leishmania infantum*)

Leishmania infantum, transmitted by phlebotomine sand flies, causes a severe form of visceral leishmaniosis in dogs in many parts of the world. If left untreated or treated at a progressive stage, leishmaniosis can be fatal. Dogs act as primary reservoirs *L. infantum* for human infection.

Parasite: *Leishmania infantum* (Note: several other species of *Leishmania* may infect dogs worldwide [1])

Common name: Leishmania

Host: Dogs, cats, humans

Incubation period: Weeks to years

Location in host: Reticuloendothelial system (phagocytic cells)

Distribution: South America, Middle East, Southern Europe, North Africa and Central Asia

Transmission route: Phlebotomine sand flies (*Lutzomyia* spp. in the Americas and *Phlebotomus* spp. elsewhere), blood transfusion, venereal and transplacental

Zoonotic: Yes

Distribution

Leishmania infantum is endemic to the Mediterranean basin, Central Asia, western China, and South America. Canine infections with other species of *Leishmania* may occur in different countries worldwide [1], including as *L. tropica*, *L. major*, *L. mexicana*, and *L. braziliensis*, which may cause mainly cutaneous manifestations in dogs.

Clinical signs

Leishmaniosis is a parasitic infection with a wide range of clinical signs. The disease may affect both visceral organs and the skin, or can manifest without skin abnormalities. Dogs and cats may present with visceral and cutaneous manifestations.

The infection outcome depends on the animal's immune system. Some dogs will eliminate the infection, some will develop subclinical infection and others will develop severe chronic disease. Dogs can present clinical signs or be infected subclinically. Clinical signs may include enlarged lymph nodes, splenomegaly, exfoliative dermatitis, nodular sores on the skin, ulcers, alopecia, conjunctivitis, blindness, epistaxis, muscular atrophy (**Fig 1a and 1b**).

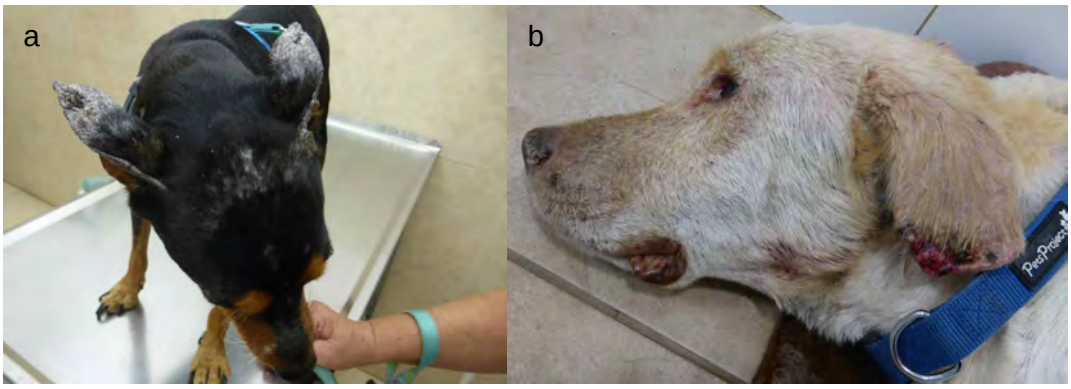

Figure 1 Dogs with clinical signs of leishmaniosis (a and b). (*Image credit: Dr. G. Baneth*)

Skin lesions include multiple ulcerative mucocutaneous lesions, ulcers on the nose, lips, testis and alopecia around eyes.

Diagnosis

Clinical diagnosis may be difficult because clinical signs are variable. Detection of amastigote forms within the cytoplasm of polymorphic nuclear cells or extracellularly in stained smears of skin lesions, bone marrow, spleen or lymph node aspirates, or other infected tissues (**Fig 2**).

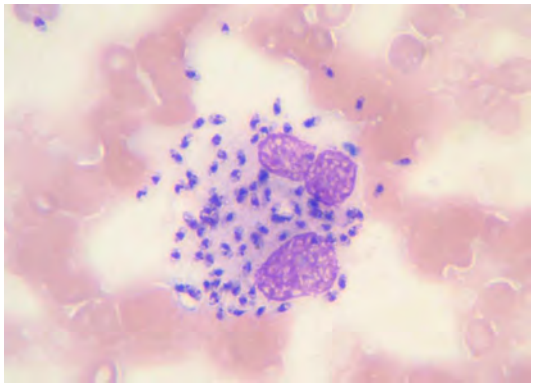

Figure 2 Intracellular and extracellular amastigotes of *Leishmania infantum* in a splenic *smear.* (*Image credit: Dr. G. Baneth*)

Serology is the most common method for diagnosis of dogs with suspected clinical signs of leishmaniosis. The immunofluorescent antibody test (IFAT), ELISA, and immunochromatographic assays are the most frequently used tests by veterinarians, although they vary in sensitivity and specificity. It is very important to consider cross-reactivity with other parasitic infections, especially with other *Leishmania* spp. and *Trypanosoma* spp. in regions where these parasites are prevalent in dogs (e.g., South America).

The polymerase chain reaction (PCR) is a very sensitive technique for the diagnosis of *Leishmania* infection but dogs may frequently be positive in dogs from areas where infection is endemic due to subclinical infection. Positive serology has a higher correlation with the presence of clinical disease. For further information, refer to the LeishVet guidelines (http://www.leishvet.org/).

Treatment

Most utilised drug protocols are:

- Meglumine antimoniate (Glucantime) – 75-100 mg/kg, SC, SID for 30 days in combination with allopurinol – 10 mg/kg, PO, BID until clinical signs are not present, haematology and serum biochemistry normalize, and serology reverts to negative.
- Miltefosine (Milteforan) – 2 mg/kg, PO, SID for 30 days in combination with allopurinol – 10mg/kg, PO, BID until all three conditions mentioned above are met.
- Allopurinol alone at 10 mg/kg PO BID in dogs with severe kidney disease or when other drugs are not available.

Control

The main and most effective way to prevent *Leishmania* infection is through the utilization of topical insecticides including collars and spot-on formulations of pyrethroids.

In countries where efficacious vaccines are marketed, vaccines can be used and started at a young age before exposure to infection. Vaccinated dogs should be negative to infection prior to vaccination and always be protected with topical insecticides.

Prophylaxis can be achieved using all protective methods available. In addition, dogs can be housed indoors from dusk to dawn, ideally in fine mesh netted environments to decrease sand fly bites.

Public health considerations

Several species of *Leishmania* have been described, most of which are zoonotic. Canines are known as the major host for *L. infantum*, in both urban and rural environments. Culling of seropositive animals practiced in some countries is controversial due to ethical issues and lack of proven effectiveness.

Reference

[1] Cantacessi C, Dantas-Torres F, Nolan MJ, Otranto D. The past, present, and future of *Leishmania* genomics and transcriptomics. *Trends Parasitol*. 2015;31:100-108.

Trypanosoma (*Trypanosoma evansi*)

Trypanosoma evansi is a protozoal pathogen closely related to African trypanosomes, which causes the disease 'Surra' in ruminants, horses and camels. Dogs are highly susceptible to *T. evansi* infection and they often exhibit severe clinical signs than can lead to death.

Parasite: *Trypanosoma evansi*

Common name: Trypanosoma

Hosts: Ruminants, horses, camels, dogs, cats

Location in host: Free in bloodstream

Distribution: Asia, Latin America, North Africa

Transmission route: Biting insects (tabanids and *Stomoxys*), iatrogenic, oral transmission

Zoonotic: Yes

Distribution

The disease is distributed in North Africa, the Middle East, Turkey, India, southern Russia, South-East Asia, Indonesia, the Philippines and Latin America.

Clinical signs

Trypanosoma evansi infection in dogs includes fever, anorexia, lethargy, lymphadenomegaly, hepatosplenomegaly, oedema, ascites, petechial haemorrhages, uveitis, oculo-nasal discharge, corneal oedema reminiscent of blue eye caused by canine adenovirus infection, and neurological signs associated with meningoencephalitis.

Diagnosis

The diagnosis of *T. evansi* trypanosomiasis involves detection of trypomastigote forms of the parasite by cytology of blood, body fluids or tissues by microscopy (**Fig 1**). Dogs may have anaemia, leucocytosis or leukopenia and thrombocytopenia. Serum biochemistry abnormalities include increased activities or liver enzymes, azotaemia, hypoalbuminemia and hyperglobulinemia. PCR with sequencing are useful for detection of low parasitaemia and for species determination. ELISA, IFA and the card agglutination trypanosomiasis test (CATT) are available for the detection of antibodies against *T. evansi*.

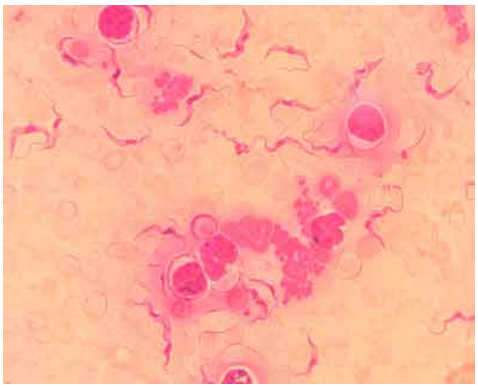

Figure 1 *Trypanosoma evansi* in a stained blood smear from an infected dog. (*Image credit: Dr. B. K. Linh*)

Treatment

Trypanosoma evansi infection in dogs can be treated with off-label use of diminazen aceturate (5 mg/kg IM) with variable responses noted. Off-label suramin (70 mg IV in 100 mL 0.9% NaCl TID every third day) lead to the resolution of parasitaemia 3 days later following the second injection in a dog weighting 8 kg [1].

Control

Disallowing consumption of raw meat and eliminating dog contact with vectors by using topical repellents and insecticides such as collars and spot-on formulations (e.g. permethrin, flumethrin, deltamethrin).

Public health considerations

Rare zoonosis. To date, five human cases of *T. evansi* infection have been reported. Livestock are considered primary reservoirs.

References

[1] Defontis M, Rochartz J, Engelmann N, Bauer N, Schwierk C, Buscher VM, Moritz A. Canine *Trypanosoma evansi* infection introduced into Germany. *Vet Clin Pathol*. 2012;41:369-374.

Standard Operating Procedures (SOP)

SOP 1: Simple Faecal Float

The simple faecal floatation procedure is suitable for the isolation and identification of a majority of nematode eggs and protozoan (oo)cysts in canine faeces. The method is quick, inexpensive and does not require use of a centrifuge.

Reagent

- Flotation solution (e.g. saturate salt or sodium nitrate)

Preparation of flotation solutions of specific gravity (S.G.) 1.20:

Sodium nitrate solution
Dissolve 315 g sodium nitrate in approximately 700 ml warmed distilled water (dH_2O). Add more dH_2O until the entire solution weighs 1200 g (this equates to a S.G. of 1.2). Mix solution and then check S.G. with hydrometer.

Saturated salt
Dissolve salt (~300-400 g depending on purity) in 1000 ml warmed dH_2O while stirring continuously. Keep adding more salt until no more dissolves (i.e. salt remains precipitated out of solution once cooled). Check S.G. with hydrometer.

Procedure

1. Place ~2 g faeces into a wide-mouthed plastic disposable cup
2. Add ~4 ml flotation solution to the jar and mix with faeces thoroughly
3. Add a further 4 ml flotation solution to the jar and mix again
4. Pour/Filter this faecal suspension through a tea strainer into a new jar
5. Empty the contents of the jar into a 10-15 ml test-tube supported in a rack or stand
6. Keep adding contents or top up with floatation solution until a positive meniscus forms over the lip of the test tube
7. Carefully place a 22 x 22 mm coverslip on top of the test tube
8. Stand for 10–15 min
9. Carefully lift off the coverslip from the tube, with the drop of fluid adhered to the bottom of it, and place it on a microscope slide
10. Examine under a light microscope at low power (10x) for helminth stages and at high power (40x) for protozoal stages

For an alternative step-by-step guide with useful images of this procedure, refer to:

http://www.rvc.ac.uk/review/parasitology/Flotation/Simple_flotation/Purpose.htm

Safety precautions

Wear lab coat and disposable gloves

Wash hands thoroughly when finished

Clean up procedures

Pour sodium nitrate into appropriate chemical waste container

Dispose of all slides and cover slips in a sharps container

Clean all equipment (tea strainer, glass test tubes) thoroughly with a 10% bleach solution

Wipe down work area with 70% ethanol

SOP 2: Centrifugal Faecal Flotation

The zinc sulfate [specific gravity (S.G.) 1.18] centrifugal flotation procedure is suitable for the isolation and identification of a protozoan cysts and oocysts in canine and feline faeces, in particular cysts of *Giardia duodenalis*. Centrifugal flotation is also more sensitive for the isolation of heavier nematode eggs such as those of *Trichuris vulpis* and *Spirocerca lupi,* in which a heavier flotation solution with a S.G. of 1.25 is utilised (e.g. Sheather's sugar solution). These methods are inexpensive; however, they do require use of a centrifuge.

Reagents

- Flotation solution (e.g., Zinc sulfate solution or Sheather's solution)
- Lugol's iodine

Preparation of flotation solutions

Zinc sulfate solution (S.G. 1.18)
Dissolve 331 g zinc sulfate in 900 ml warmed distilled water (dH_2O). Add more dH_2O until the entire solution weighs 1180 g (this equates to a S.G. of 1.18). Mix solution and then check S.G. with hydrometer. Note: if zinc sulfate heptahydrate is used, then additional quantities will be needed (e.g., approx. 750 g).

Sheather's solution (S.G. 1.25)
To 355 ml hot water, add (while stirring) 454 g sugar. Add 6 ml formalin per 454 g sugar. Adjust to ensure S.G. is 1.25 using a hydrometer.

Procedure

1. Place ~2 g faeces into a wide-mouthed plastic disposable cup
2. Add ~4 ml flotation solution to the jar and mix with faeces thoroughly
3. Add a further 4 ml flotation solution to the jar and mix again
4. Pour/filter this faecal suspension through a tea strainer into a new jar
5. Empty the contents of the jar into a 10-15 ml test-tube supported in a rack or stand
6. Centrifuge at 500 *g* for 10 min
7. Carefully add more flotation solution until a positive meniscus forms at the top of the test tube and place a 22 x 22 mm coverslip on top
8. Stand for a further 5-10 minutes
9. Carefully lift the coverslip with the drop of fluid adhered to the bottom of it and place it on a microscope slide. Adding a drop of Lugol's iodine to the slide before placing the coverslip on it can make the *Giardia* cysts easier to see
10. Examine under a light microscope at low power (10x) for helminth stages and at high power (40x) for protozoal stages

Safety precautions

Wear lab coat and disposable gloves

Wash hands thoroughly when finished

Clean up procedures

Pour zinc sulfate into appropriate chemical waste container

Dispose of all slides and cover slips in a sharps container

Clean all equipment (tea strainer, glass test tubes) thoroughly with a 10% bleach solution

Wipe down work area with 70% ethanol

SOP 3: Baermann Technique

The Baermann technique is suitable for the isolation and identification of larvae in fresh faeces (e.g. *Strongyloides* spp., lungworms)

Reagents

- Distilled water (dH_2O)

Equipment set up

Secure a glass or plastic funnel to a stand and connect a rubber tube with a clamp to the stem of the funnel.

Procedure

1. Place 3-5 g of faeces in the centre of a large cheese cloth and tie with a rubber band or string to form a pouch
2. Place this within a tea strainer and suspend this in the funnel or within the mouth of a 50 ml centrifuge tube using toothpicks to keep the faecal pouch in place
3. Add warmed dH_2O to the funnel until the water covers the top of the faecal pouch
4. Leave standing for 24 h
5. If utilising a funnel, open the stopper on the rubber tubing and collect 2 ml of the filtered sediment into a test tube. If using a 50 ml centrifuge tube, go to step 7
6. Leave the test-tube standing for 30 min, or alternatively centrifuge at 500-1000 *g* for 2 min
7. Carefully remove the supernatant with a pipette, leaving ~0.5 ml of the sediment undisturbed
8. Take 1-2 drops of the sediment and place on a microscope slide with a cover slip
9. Examine under a light microscope at low power (10x) for larvae

For an alternative step-by-step guide with useful images of this procedure, refer to: http://www.rvc.ac.uk/review/parasitology/Baermann/Purpose.htm

Safety precautions

Wear lab coat and disposable gloves

Wash hands thoroughly when finished

Clean up procedures

Dispose of all slides and cover slips in a sharps container

Clean all equipment (tea strainer, glass test tubes) thoroughly with a 10% bleach solution

Wipe down work area with 70% ethanol

SOP 4: Sedimentation Technique

The faecal sedimentation technique is suitable for the isolation and identification of heavier eggs, especially those of flukes (e.g. *Paragonimus* spp.). The method is quick, inexpensive and does not require the use of a centrifuge.

Reagents

- Distilled water (dH_2O)
- 5% aqueous methylene blue solution

Procedure

1. Soak 5 g faeces in 50 ml dH_20 and mix thoroughly
2. Pass through tea strainer into a plastic jar to filter
3. Pour all contents into a conical test tube (50 ml)
4. Allow to sediment for 5 min
5. Pour off supernatant
6. Pour sediment into a 10-15 ml conical test tube
7. Allow to sediment 5 min
8. Pour off supernatant carefully
9. Can add 1 or 2 drops of 5% aqueous methylene blue solution in test tube to aid in identification (yellow or colourless fluke eggs against a blue background)
10. Transfer 1-2 drop of the sediment to a microscope slide, place a cover slip and examine using a light microscope at low power (4x and 10x)

Safety precautions

Wear lab coat and disposable gloves

Wash hands thoroughly when finished

Clean up procedures

Dispose of all slides and cover slips in a sharps container

Clean all equipment (tea strainer, glass test tubes) thoroughly with a 10% bleach solution

Wipe down work area with 70% ethanol

SOP 5: Modified Knott's Test

The method is used for the detection of microfilariae in the blood. The method is more sensitive than a direct smear with fresh blood as it concentrates the microfilariae.

Reagents

- 2% formalin
- 1% methylene blue

Procedure

1. Mix 1 ml blood with 9 ml of 2% formalin in a conical centrifuge tube
2. Invert the tube gently 4 times to mix the solution
3. Centrifuge at 500 *g* for 5 min
4. Discard supernatant
5. Stain sediment for 1-2 min with 1-2 drops of 1% methylene blue
6. Add a drop of the sample on a glass slide and cover with a coverslip
7. Examine the slide under a light microscope at low power (10x) for microfilariae

Safety precautions

Wear lab coat and disposable gloves

Clean up procedures

Dispose of all slides and cover slips in a sharps container

SOP 6: Acid Fast Stain for *Cryptosporidium* oocysts

As the oocysts of *Cryptosporidium* spp. are very small and difficult to detect by inexperienced examiners, this method provides specific staining and allows an easier detection.

Reagents

- Absolute methanol
- Kinyoun's carbol fuchsin
- 10% sulfuric acid solution (H_2SO_4)
- 3% Malachite green

Procedure

1. Make a thin faecal smear and allow to air dry
2. Fix with absolute methanol for 10 min and allow smear to dry
3. Stain with cold Kinyoun's carbol fuchsin strong stain (filtered) for 5 min
4. Wash thoroughly in tap water until no further stain comes out (very important step that can take 3-5 min)
5. Decolourise in 10% H_2SO_4 (for very thin smears a rapid dip in Coplin jar of acid followed by an immediate rinse in tap water is sufficient)
6. Counterstain with 3% Malachite green for 2-5 min
7. Wash in tap water and blot dry
8. Examine under a light microscope at high power (40x) for oocysts

Results

Oocysts are seen as acid fast (bright pink) oval to round bodies (4 to 6 μm in diameter), surrounded by a colourless halo. Bacteria and yeasts stain green.

Safety precautions

Wear lab coat and disposable gloves

Wash hands thoroughly when finished

Clean up procedures

Dispose of all disposable equipment in clinical waste bin or sharps as appropriate