

GUIDELINE for Lungworm disease

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Synopsis

Cardiopulmonary nematodes are emerging parasites of dogs and cats in Europe which have received growing attention by researchers in recent years. Significant progress has been made, mainly in the diagnosis and treatment of infection.

Aelurostrongylus abstrusus (Strongylida, Angiostrongylidae) is the best-known feline lungworm and is regarded as the most prevalent worldwide in domestic cats. Other lungworms in the cat include *Oslerus rostratus*, *Troglostrongylus brevior*, *Capillaria aerophila* and *Paragonimus spp.*; *A. abstrusus*, *O. rostratus* and *T. brevior* may cause mixed infections as they share the same intermediate and paratenic hosts.

Lungworm infections may be asymptomatic, or cause mild to severe respiratory signs due to bronchopneumonia, sometimes complicated by pleural effusion or pneumothorax, and cats show nasal discharge, tachypnoea, dyspnoea and/or coughing. The disease can be fatal. Kittens may be vertically infected and develop more severe disease at an early age, due to the smaller diameter of their respiratory tracts and their immature immune system. It is advisable to investigate for the presence of lungworm infection in cases with right-sided heart disease associated with signs of pulmonary hypertension in outdoor cats. Uncared-for outdoor cats, such as those included in trap-neuter-release programs, are at a higher risk for lungworm infection.

The Baerman migration method is considered the enrichment technique of choice for diagnosis, but takes 24 hours to perform and false negatives may occur. The major limitation of copromicroscopy is the inability to diagnose infection in the pre-patent period, which lasts about 1-2 months. PCR assays specific for *A. abstrusus*, *T. brevior*, and *C. aerophila* have been validated.

Fenbendazole paste, and spot on formulations of fipronil 8.3 % / (S)-methoprene 10 % / eprinomectin 0.4 % / praziquantel 8.3 % or imidacloprid 10 % / moxidectin 1 % are licenced for the treatment of aelurostrongylosis. The spot on formulation containing eprinomectin is licenced also against L3 and L4 forms and also for troglostrongylosis. Other oral drugs (milbemycin oxime/praziquantel tablet) or spot on formulations (emodepside 2.1 % / praziquantel 8.6 %, selactamin) were however found effective against lungworms and the latter is licenced for treating capillariosis. In severe cases intensive medical care is needed and broad-spectrum antibiotics should be given together with corticosteroids.

Capillaria aerophila has zoonotic potential and sporadic cases of human capillariosis have been described, causing a productive cough, haemoptysis and lung lesions.

Agents

Infection of the lower respiratory tract can be caused by a number of parasitic nematodes. Certain metastrongyloid worms are commonly defined as lungworms because the adult stage is located in the lungs of their hosts, but actually some trichuroids and flukes also live in the respiratory system (Bowman, 2000; Conboy, 2009; Traversa et al., 2010; Traversa and Di Cesare, 2013). According to recent investigations, lungworms are the most prevalent parasites (10.6 %) detected in European cats after ascarids (16.5 %) and *Aelurostrongylus abstrusus* (Strongylida, Angiostrongylidae) is the most well-known and the most common (Giannelli et al., 2017). It is small in length (5-10 mm) and very narrow (less than 100 µm) and capable of colonising the respiratory bronchioles and alveolar ducts of domestic cats and other felids worldwide (Pennisi et al., 1995; Traversa et al., 2010). Other respiratory mollusc-borne

metastrongyloids are commonly reported at necropsy in wild felids but they were considered rare in domestic cats. *Troglostrongylus* spp. (Strongylida, Crenosomatidae) are reported in wild felids but *Troglostrongylus brevior* is increasingly detected in domestic cats (Fitzsimmons, 1961; Jefferies et al., 2010; Brianti et al., 2012, 2013, 2014a; Tamponi et al., 2014). Adults are slightly bigger (7-17 mm in length and 0.2-0.4 mm wide) than *A. abstrusus* worms and are located in the bronchi and bronchioles (Brianti et al., 2012; Giannelli et al., 2014a). *Oslerus rostratus* (Strongylida, Filaridae) exceeds 30-40 mm in length and infects the bronchial submucosa, mainly in wild cats such as bobcats or in feral cats (Juste et al., 1992; Bowman, 2000; Millan and Casanova, 2009; Jefferies et al., 2010; Brianti et al., 2014b). The trichuroid *Capillaria aerophila* (syn. *Eucoleus aerophilus*) has a low host-specificity and it is not uncommon in dogs and cats as well as wild carnivores (Traversa et al., 2009a). It is also a zoonotic parasite causing a potentially severe pulmonary disease in humans (Lalosević et al., 2008). *Capillaria aerophila* is found in the submucosa of the trachea, bronchi and bronchioles (Traversa et al., 2009a, 2010). Mixed infections caused by respiratory nematodes are often reported and both *T. brevior* and *O. rostratus* are frequently seen together (Juste et al., 1992; Risitano et al., 2008; Jefferies et al., 2010; Di Cesare et al., 2014a; Tamponi et al., 2014; Varcasia et al., 2014a; Giannelli et al., 2017). These infections were probably misdiagnosed as *A. abstrusus* in the past because of morphometric similarities of their larval stages in faeces (L1), but a spill-over from wild reservoirs cannot however be excluded and it is supported by the occurrence of the same parasite mitochondrial haplotype in wild and domestic cats (Traversa and Di Cesare, 2013; Brianti et al., 2014a; Traversa et al., 2017; Crisi et al., 2018). *Angyostrongylus chabaudi* (Strongylida, Angiostrongylidae) is a slender and elongated (15-24 mm long and 180-300 µm wide) metastrongylid heartworm of the European wildcat rarely reported in the proximal pulmonary arteries of domestic cats in Italy (Varcasia et al., 2014b; Traversa et al., 2015). *Paragonimus* spp. are lung flukes reported in many animals, including cats and humans, and some species are of zoonotic concern. Many species are found in cats, including *P. kellicotti*, and 1-10 adults measuring 8-18 mm x 4-8 mm live in subpleural cysts or bullae (Conboy, 2009).

Life cycle and transmission

Aelurostrongylus abstrusus, *O. rostratus* and *T. brevior* all have an indirect life cycle involving terrestrial molluscs. Eggs laid by female worms hatch in the respiratory tract and L1 larvae are coughed up, swallowed and eliminated in the environment with the faeces. They can actively enter slugs or snails where they moult into the infectious L3 stage (López et al., 2005; Di Cesare et al., 2013; Jezewski et al., 2013; Giannelli et al., 2014b; Valente et al., 2017). The biological cycle in the intermediate host is influenced by environmental temperature: a higher rate of larval development is observed at warmer temperatures (Di Cesare et al., 2013). Slugs and snails are not usually predated or ingested by cats, but infective larvae of *A. abstrusus* and *T. brevior* are shed in the mucus of snails and they are found in water where infected gastropods may die submerged (Giannelli et al., 2015). This means that drinking water from outdoor water bowls could be a source of cat infection. The L3 larvae of *A. abstrusus* are also found in a wide range of paratenic hosts (rat, mouse, lizard, frog, birds) commonly predated by cats (Bowman, 2000; Conboy, 2009; Jezewski et al., 2013; Colella et al., 2019). Recently, also cockroaches (*Periplaneta americana*) were proposed as further paratenic hosts (Falsone et al., 2017). The role of paratenic hosts is not known in the epidemiology of *O. rostratus* and *T. brevior*. The ingestion of L3 by the cat is the most recognized means of transmission of lungworms but vertical transmission via the placenta or milk cannot be excluded, as adult egg-laying worms have been found in kittens as young as eight weeks of age (Tamponi et al., 2014). An experimental study demonstrated that egg production starts 4-6 weeks after infection and may last for months, although it can be irregular (Hamilton, 1968a; Barrs et al., 1999; Pennisi et al., 1995; Dirven et al., 2012; Schnyder et al., 2014). Vertical transmission of *T. brevior* was observed in a queen and patent infections were detected in about one-month old kittens, however it is not clear if transmission occurs via the placenta or the milk (Brianti et al., 2013; Diakou et al., 2014; Tamponi et al., 2014; Traversa et al., 2018). *Troglostrongylus brevior* and *A. abstrusus* larvae may develop simultaneously in the same mollusc host (*Helix aspersa*) and overwinter for at least 120 days (Giannelli et al., 2014b). The molluscs involved in the life cycle of *A. chabaudi* are not known. *Capillaria aerophila* has a direct cycle and eggs laid by female worms in the respiratory tract are swallowed and reach the environment in the faeces. After 30-45 days, embryonated eggs become infective when ingested by cats. Earthworms are facultative paratenic hosts (Traversa et al., 2009a). When cats ingest infective eggs or earthworms carrying larvae, the larvae migrate to the lung and develop into the adult stage in 3-6 weeks (Anderson, 2000). The life cycle of *Paragonimus* spp. is associated with freshwater environments and is complex as it involves two intermediate hosts. Motile miracidia are freed from eggs when swallowed and then passed in faeces from infected cats and penetrate aquatic snails; cercarial stages developed in snails will move from them, actively entering the second intermediate host (crab or crayfish). Cats are infected after eating the second intermediate host where metacercariae finally develop. Young flukes develop from metacercariae in the cat intestine, cross the intestinal wall and the diaphragm to the pleural cavity where they penetrate the lung parenchyma and become reproducing adults in about 6 weeks (Conboy, 2009).

Epidemiology

Feline lungworm infection is worldwide receiving increasing attention; in a recent multicentric study involving 1990 cats in 12 European countries, lungworms L1 were found in 0.8-35.8 % of freshly passed faeces of cats (Giannelli et al., 2017). *A. abstrusus* is the most

frequently detected species not only in Europe, but also in the Americas, Asia and Australia (Gregory and Munday, 1976; Coman et al., 1981; Mundim et al., 2004; Abu-Madi et al., 2007; Conboy, 2009; Di Cesare et al., 2011; Lucio-Forster and Bowman, 2011; Echeverry et al., 2012; Kohart et al., 2014; Philbey et al., 2014; Tamponi et al., 2014; Hawley et al., 2016; Hansen et al., 2017; Giannelli et al., 2017; Grandi et al., 2017; Soares et al., 2017; Penagos-Tabares et al., 2018; Gueldner et al., 2019; Zottler et al., 2019). Prevalence rates vary and endemicity is linked to climatic and ecological factors that may influence: a) the vitality and developmental capacity of L1 larvae; b) the presence of suitable intermediate hosts in the environment; c) the number of days needed for the development of the infective stage (L3).

The diagnostic method used in epidemiological studies and the characteristics of the population investigated heavily influence the results obtained (Lacorcia et al., 2009; Traversa et al., 2008a, 2010; Lucio-Forster and Bowman, 2011). Feral and free-roaming cats are at a higher risk because of their predator activity, as are cats with respiratory signs and young cats (Iorio and Traversa, 2008; Barutzky and Schaper, 2013; Giannelli et al., 2017). In Tirana (Albania), *post mortem* examination of the lungs of 18 feral cats revealed that nine (50 %) were positive for *A. abstrusus* (Knaus et al., 2011). Using a low-sensitive diagnostic method, such as faecal standard flotation technique, a prevalence rate of 1-25 % is obtained in a general cat population (Miró et al., 2004; Brianti et al., 2008; Mircean et al., 2010; Barutzky and Schaper, 2011; Tamponi et al., 2014). Recently, mass serological screenings were performed in Switzerland and Italy by ELISA technique to evaluate exposure of cats to the parasite. In Switzerland 10.7 % of about 4000 cats were positive (Gueldner et al., 2019) while in Italy overall 9 % of exposure was evidenced in about 1000 cats, with higher positivity in the South (22.5 %) of the country compared to the North (7.2 %) or the Centre (5.3 %) (Cavalera et al., 2019).

T. brevior infection was reported in cats from Spain, Italy, Greece, Cyprus, and Bulgaria (Jefferies et al., 2010; Brianti et al., 2012, 2013; Annoscia et al., 2014; Diakou et al., 2014; Di Cesare et al., 2014a, 2015; Giannelli et al., 2014a; Varcasia et al., 2014a). The first epidemiological data on *T. brevior* in domestic cats were provided in 2014 in Sardinia (Italy) where 6.5 % of a sample of 107 cats tested positive compared to 25.2 % that tested positive for *A. abstrusus* (Tamponi et al., 2014). Afterwards, *T. brevior* was detected in 5.6 % of cats from areas of Greece where wildcats are not present confirming that *Troglostrongylus* is not a negligible lungworm of domestic cats (Diakou et al., 2015). Moreover, in Cyprus (5 %) and in Central and Southern Italy (8.7 %) *T. brevior* infections were found to be more prevalent than *aelurostrongylosis* (2.0 % and 4.7 % respectively; Diakou et al., 2017; Cavalera et al., 2018). In European cats with regular outdoor access, *A. abstrusus* positivity was 8.2 % with the Baerman test, while in the same population *T. brevior* prevalence was 2.0 %, *C. aerophila* 1.6 % and *O. rostratus* 0.4 % (Giannelli et al., 2017). Mixed infections were observed among the three more prevalent species and about half of the cats with *T. brevior* or *C. aerophila* infections were affected by mixed infections (Giannelli et al., 2017). *A. chabaudi* is rarely reported in the domestic cat in Italy, even as mixed infections with *A. abstrusus* and *T. brevior* (Varcasia et al., 2014b; Traversa et al., 2015).

O. rostratus is considered an uncommon parasite in domestic cats, but the prevalence in feral cats was found to be 24 % on Majorca (Spain). Infection was also reported in a cat in Northern Spain (Juste et al., 1992; Millan and Casanova, 2009). The incidental occurrence of a few adult *O. rostratus* worms was reported in Sicily (Italy) during the necropsy of an adult cat that had died following a road traffic accident and in one cat out of 575 (0.2%) investigated in Central and Southern Italy (Brianti et al., 2014b). *Capillaria aerophilae* has a sporadic occurrence in cats, dogs and humans in Europe. In Central Italy, a prevalence of 3-14 % was found in the feline population and *C. aerophila* was the respiratory nematode with the widest geographic distribution (Traversa et al., 2009a, 2010; Di Cesare et al., 2011; Traversa et al., 2019a). Recently a clinical case was reported in France (Elhamiani Khatat et al., 2016). The recent development of molecular PCR assays specific for mollusc-borne feline lungworms sharing the same ecological niches, as well as for *C. aerophila*, is likely to be of great value for epidemiological investigations, overcoming the difficulties of copromicroscopy for differentiating the metastrongylid L1 (Traversa et al., 2008b; Jefferies et al., 2010; Di Cesare et al., 2012; Traversa and Di Cesare, 2013; Annoscia et al., 2014).

Paragonimus spp. infections are reported in cats from the Americas, Africa and Asia (Sohn and Chai, 2005; Conboy, 2009; Foster and Martin, 2011). Paragonimiasis is most prevalent in cats and dogs in some parts of Asia (Liu et al., 2008).

Prevalence rates and the occurrence of case reports for *A. abstrusus*, *C. aerophila*, *O. rostratus* and *T. brevior* in European countries are shown in table 1.

Table 1: Prevalence rates and occurrence of case reports for *A. abstrusus*, *C. aerophila*, *O. rostratus* and *T. brevior* in European countries

	<i>A. abstrusus</i>	<i>T. brevior</i>	<i>C. aerophila</i>	<i>O. rostratus</i>	References
Albania	50 %	-----	-----	-----	1)
Belgium	0.9 %	-----	0.9 %	-----	2)
Bulgaria	27.5-33.3 %	10.8 %	10.8 %	-----	2) 3)

Croatia	22 %	-----	-----	-----	4)
Cyprus	2 %	5 %	-----	-----	5)
Denmark	0-31.4 %	-----	3.1 %	-----	6)
France	4.4 %	-----	1 %	2.2 %	2) 7)
Germany	0.7-6.5 %	-----	0.2 %	-----	8) 9) 10)
Greece	8.5 %	5.6 %	4.2 %	-----	2) 11)
Hungary	14.5-22.5 %	-----	3.8 %	2.5 %	2) 12) 13)
Italy	1.2-25.2 %	0-18 %	0-14.3 %	0-0.8 %	2) 14) - 31)
Netherlands	2.6 %	-----	-----	-----	32)
Portugal	1.7-17.4 %	-----	0-1.6 %	-----	2) 33) 34)
Romania	5.6-14.2 %	-----	0.7-6.5 %	-----	2) 35)
Spain	5 %	3 %	1.3 %	1-24 %	2) 36) 37)
Sweden	0.5 %	-----	-----	-----	38)
Switzerland	0.8-2.3 %	-----	4.7 %	-----	2) 7) 39)

In red colour are values of prevalence >20 %, in blue colour values between 5 and 20 %, in black colour values <5 %; ----- = no data

References: 1) Knaus et al., 2011; 2) Giannelli et al., 2017; 3) Stoichev et al., 1982; 4) Grabarević et al., 1999; 5) Diakou et al., 2017; 6) Hansen et al., 2017; 7) Traversa et al., 2010; 8) Taubert et al., 2009; 9) Becker et al., 2012; 10) Barutzky and Schaper, 2013; 11) Diakou et al., 2014; 12) András and Péter, 2002; 13) Capari et al., 2013; 14) Grandi et al., 2005; 15) Brianti et al., 2008; 16) Risitano et al., 2008; 17) Pennisi et al., 1995; 18) Brianti et al., 2012; 19) Mugnaini et al., 2012; 20) Brianti et al., 2013; 21) Riggio et al., 2013; 22) Spada et al., 2013; 23) Annoscia et al., 2014; 24) Brianti et al., 2014b; 25) Di Cesare et al., 2014a; 26) Varcasia et al., 2014a; 27) Di Cesare et al., 2015; 28) Traversa et al., 2015; 29) Cavalera et al., 2018; 30) Traversa et al., 2018; 31) Traversa et al., 2019a; 32) Robben et al., 2004; 33) Payo-Puente et al., 2008; 34) Soares et al., 2017; 35) Mircean et al., 2010; 36) Miró et al., 2004; 37) Jefferies et al., 2010; 38) Grandi et al., 2017; 39) Zottler et al., 2019

Pathogenesis

The severity of lesions depends on the worm species and burden. Kittens also seem to develop more severe disease (Barrs et al., 1999; Grandi et al., 2005; Risitano et al., 2008; Dirven et al., 2012). This may be explained by their smaller lung volume and the small diameter of their trachea and bronchi, which are more easily blocked by worms, in particular by *T. brevior*. Their immature immune system also seems to facilitate infection: experimental re-infection of kittens with *A. abstrusus* L3 larvae about one year after the initial symptomatic infection failed to induce respiratory signs or lung lesions (Hamilton, 1968a). In cats with natural aelurostrongylosis, the more severe radiologic abnormalities and the higher larval burdens were found in younger animals (Genchi et al., 2014) (Fig. 1).



Fig. 1. Right lateral thoracic radiograph of a kitten affected by severe aelurostrongylosis showing a diffuse focal alveolar pattern. Courtesy of Maria Grazia Pennisi, Department of Veterinary Sciences, University of Messina, Italy

An infectious dose of *A. abstrusus* larvae of <100 L3 does not induce clinical signs but infective doses of 800 to 3200 larvae severely affect the lung and may even be lethal (Hamilton, 1967; Dennler et al., 2013). However, at normal infective doses, the individual immune response significantly affects the parasite life cycle (Schnyder et al., 2014). Cats repeatedly infected with a low number of larvae do not develop clinical disease when challenged with a high dose (Hamilton, 1969). The role of immunity is also confirmed by the fact that passive immunity protects experimentally infected kittens and can help some cats halt the parasite life cycle in the lung by preventing the patent phase of infection (Hamilton, 1968b; Schnyder et al., 2014). It has been known for a long time that eosinophilia is evident 2-6 weeks after the ingestion of L3 larvae of *A. abstrusus* and immune-mediated reactions of I, III and IV type are associated with alveolar, interstitial, peribronchial and vascular lesions, and may lead to the death of parasites several months later (Hamilton, 1966; Conboy, 2009). A more recent experimental study provides more detailed information on the clinical signs, haematology, biochemistry, coagulation analysis, computed tomography, coprology and *post mortem* examination in young adult cats (Dennler et al., 2013; Schnyder et al., 2014). Infected cats had moderate, non-specific clinical signs (fever, apathy, weight loss, lymph node enlargement) and respiratory signs (dyspnoea, respiratory sounds, cough). Leucocytosis, massive and persistent eosinophilia and, in some cases, severe lymphocytosis were the most frequently observed abnormalities but no changes were detected on serum biochemistry. Various coagulation abnormalities were found, with a frequent occurrence of low fibrinogen values suggesting increased consumption. Dose-dependent imaging changes in the thorax consisted of pulmonary nodules, a bronchial pattern and lymphadenomegaly, and were found even in a cat that did not develop a patent infection (Dennler et al., 2013). *Aelurostrongylus abstrusus* eggs accumulate in the alveoli and bronchioles, inducing an inflammatory reaction in the lung (Fig. 2).

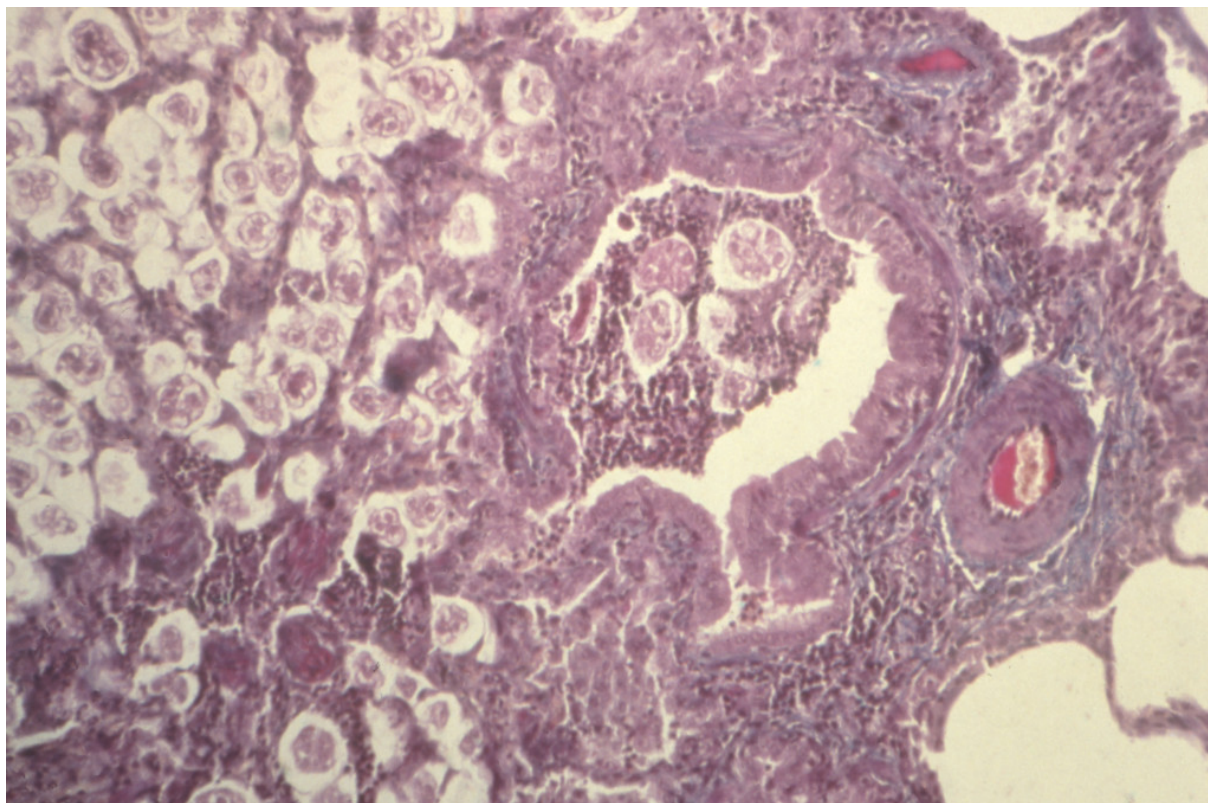


Fig. 2. Alveolitis with larval accumulation, bronchiolitis and bronchiectasis in the lung of a cat affected by aelurostroglyosis (haematoxylin-eosin stain). Courtesy of Maria Grazia Pennisi, Department of Veterinary Sciences, University of Messina, Italy

Multiple subpleural nodules (Fig. 3) are caused by the granulomatous reaction surrounding clusters of eggs and adult worms and emphysema is due to parasitic accumulation in the alveolar spaces. Bronchitis is severe and diffuse, usually manifested by bronchial and peribronchial lymphoid hyperplasia, hypertrophy of the smooth muscle layer and mucosal hyperplasia with increased mucous cell secretion in the bronchi. Vascular and perivascular changes are also seen with hypertrophy and hyperplasia of pulmonary arteriolar smooth muscle and subendothelial fibrosis associated with eosinophilic infiltrates, endothelial and perivascular hyperplasia. Pulmonary hypertension may arise as a consequence of lung disease and arteriolar and bronchial changes may persist after the parasite dies, mimicking the changes found in feline asthma (Hamilton, 1966; Jonas et al., 1972; Naylor et al., 1984; Dirven et al., 2012).



Fig. 3. Multifocal subpleural nodules and haemorrhages in a severe case of aelurostrongylosis. Courtesy of Maria Grazia Pennisi, Department of Veterinary Sciences, University of Messina, Italy

Bacterial complication is frequent and can be associated with pleural effusion (Barrs et al., 1999). *Salmonella typhimurium*, *Pseudomonas* spp. and *Escherichia coli* have been isolated in some cases and infection with enteric bacteria probably results from larvae migrating from the intestine (Foster et al., 2004; Foster and Martin, 2011).

In a kitten with severe pulmonary aelurostrongylosis, enteritis and mild diarrhoea were associated with the presence of a high number of L1 larvae invading the mucosa of the small intestine (Philbey et al., 2014).

Lethal *T. brevior* infection was associated, in three kittens, with catarrhal bronchitis occluding the lumen together with the adult worms and multifocal pulmonary haemorrhages, consolidation and emphysematous foci (Brianti et al., 2012; Giannelli et al., 2014a). Irreversible pulmonary hypertension was reported in some kittens (Crisi et al., 2015).

Oslerus rostratus does not seem to be associated with severe pathologic changes in domestic cats, as few adult worms are found embedded in bronchial or peribronchial tissues inside pseudo-cysts (Juste et al., 1992; Brianti et al., 2014b).

The pathogenic potential of *A. chabaudi* is not clear and there is no evidence of parasite mating and L1 shedding in cats with angiostrongylosis (Varcasia et al., 2014b; Traversa et al., 2015).

Capillaria aerophila usually induces chronic bronchitis but infection can be asymptomatic (Holmes and Kelly, 1973; Traversa et al., 2009a). The penetration of *Paragonimus* spp. in the lung is associated with haemorrhagic foci, usually in the diaphragmatic lobe. Fluke cysts communicate with bronchi and may evolve into bullae, with a risk of developing pneumothorax.

Clinical signs

Most publications describe *A. abstrusus* infections, but it has been suggested that infection or co-infections caused by other metastrongyloids may have been erroneously reported as *A. abstrusus* infections because of difficulties with the morphometric differentiation of L1 larvae (Jefferies et al., 2010; Traversa and Di Cesare, 2013; Brianti et al., 2014a). Genetic characterization of larvae

now offers new insights and allows more accurate diagnoses.

Lungworm infections may be asymptomatic, or cause mild to severe respiratory signs due to bronchopneumonia, sometimes complicated by pleural effusion or pneumothorax (Miller et al., 1984; Barrs et al., 1999; Grandi et al., 2005; Mooney et al., 2012). A productive cough is therefore the main clinical sign, together with a mucopurulent nasal discharge, tachypnoea, dyspnoea with laboured, abdominal breathing. Increased vesicular breath sounds, wheezing and end-inspiratory crackles can be found on auscultation. In more severe cases, there is lethargy and anorexia and respiratory failure causes cyanosis and respiratory acidosis (Risitano et al., 2008; Traversa et al., 2008a; Jefferies et al., 2010; Yildiz et al., 2011; Dirven et al., 2012; Crisi et al., 2017).

Diagnostic imaging by thoracic radiography or computed tomography shows interstitial (reticular or nodular), bronchial or mixed patterns during the patent phase more frequently than alveolar or vascular changes (Crisi et al., 2017; Febo et al., 2019). A nodular pattern is usually multifocal, apart from in (Febo et al., 2019). Bronchial abnormalities may persist after clearing infection and should be differentiated from other chronic bronchial disease such as asthma (Losonsky et al., 1983; Payo-Puente et al., 2008). Moreover, imaging changes may be evident even before the phase of disease and their severity may not be related to severity of clinical signs (Dennler et al., 2013; Schnyder et al., 2014; Crisi et al., 2017; Febo et al., 2019). Subclinically infected cats may have radiographic and mild-to-moderate abnormalities (particularly in the caudal lobes) are only seen in ventro-dorsal or dorso-ventral projections of radiography or with high-resolution computed tomography (Febo et al., 2019).

Right side cardiomegaly, associated with eccentric hypertrophy and secondary to pulmonary hypertension, has been described in two kittens affected by a severe bronchopneumonia caused by *A. abstrusus* (Dirven et al., 2012). Both kittens presented with heart murmurs with maximum intensity on the right hemithorax due to tricuspidal and pulmonary regurgitation. One of the kittens died, but in the surviving kitten the heart murmur disappeared several months after the parasitological and clinical cure. Echo-doppler examination confirmed the resolution of pulmonary hypertension (Dirven et al., 2012). It is therefore advisable to investigate the presence of lungworm infection in cases of right heart disease associated with signs of pulmonary hypertension in outdoor cats. In a study of 54 cats, that died during anaesthesia in spay-neutering programs in USA, 9 % of *post mortem* investigations revealed the presence of *A. abstrusus* (Gerdin et al., 2011). Uncared-for outdoor cats, such as those included in trap-neuter-release programs, are at a higher risk of lungworm infection.

Mild normocytic, normochromic anaemia is the most common clinicopathological finding, probably due to the chronic inflammation. Eosinophilia is not found systematically in cell blood counts or in bronchoalveolar lavage (BAL) cytology (Foster et al., 2004; Reinhardt et al., 2004; Grandi et al., 2005; Risitano et al., 2008; Crisi et al., 2017). It seems that eosinophilia is influenced by the duration of infection and it is seen in the first weeks or months post infection (Schnyder et al., 2014).

Trogostrongylus spp. was considered the cause of death of parasitized kittens presenting with coughing and severe respiratory failure at diagnosis, but cases of asymptomatic infection have also been reported (Brianti et al., 2012, 2013; Di Cesare et al., 2014a, 2014b; Giannelli et al., 2014a; Crisi et al., 2017).

Capillaria aerophila infection may induce coughing (mostly dry cough), sneezing and wheezing in cats, but asymptomatic carriage is often reported (Traversa et al., 2009a, 2012; Febo et al., 2019).

Mixed infections are increasingly reported and they may cause a more severe clinical picture or poorer outcome (Traversa et al., 2012; Di Cesare et al., 2014a, 2014b; Crisi et al., 2017; Febo et al., 2019).

Diagnosis

L1 larvae are very active in the faeces and are readily detected in fresh faecal samples. Care should be taken to prevent soil contamination, as the presence of free-living nematodes may lead to misdiagnosis by an unskilled observer. L1 can be observed in direct faecal smears or by the floatation technique. In the latter method, the high specific gravity of concentrated salt or sugar solutions may induce osmotic damage to the larvae, making identification difficult (Conboy, 2009). The Baerman migration method (fig. 4 and table 2) is considered the enrichment technique of choice for metastrongyloid lungworms, and is based on the positive hydro-thermo tropism observed for live nematode larvae (Willard et al., 1988; Traversa et al., 2008a; Lacorcchia et al., 2009). It can provide quantitative information on the number of larvae found in each gram of faeces, which correlates well with the severity of the disease (Brianti et al., 2008; Genchi et al., 2014). However, dehydration of faeces occurs within a few hours of defaecation, particularly when faeces are shed into litter, and this affects L1 survival and the sensitivity of the Baerman test (Abbate et al., 2018). Moreover, it takes 24 hours to obtain results and negative tests need to be confirmed twice more for the best sensitivity.

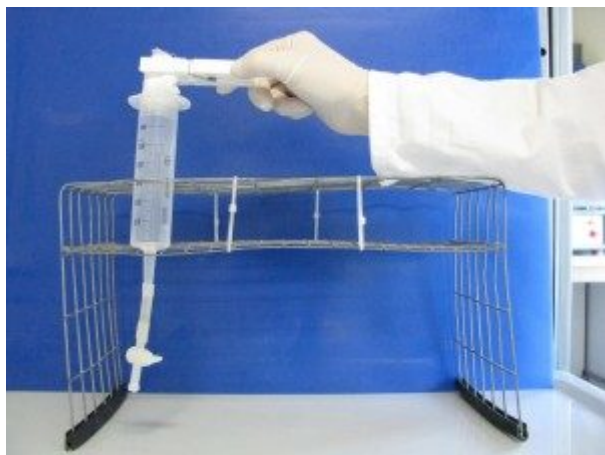


Fig. 4a: Baerman apparatus. Courtesy of Emanuele Brianti, University of Messina, Italy



Fig. 4b: Baerman apparatus. Courtesy of Emanuele Brianti, University of Messina, Italy

Table 2: The Baerman method

The Baermann method separates live larvae from a faecal sample as they are attracted by humidity (hydrotropism). It can be performed using an in-house system (Figs. 4a and 4b).

- ❖ Fill a large (60 ml) syringe with tap water
- ❖ Connect the cone of the syringe to a rubber tube, which is clamped at the end
- ❖ Orientate the syringe vertically
- ❖ Fill a cheesecloth pouch with approximately 5-10 g of faeces
- ❖ Clamp the pouch and dip it in the water-filled syringe (Fig. 4a)
- ❖ After 24 h any live larvae will have passed into the water and sedimented at the bottom of the system
- ❖ Collect a few millilitres of the water in a tube (Fig. 4b) and centrifuge (400 g x 2 mins).
- ❖ Discharge the supernatant and put one drop of the sediment fluid on a microscope slide. Cover with a coverslip and examine under a microscope at x 100 magnification.

A parasitological diagnostic device for multivalent quantitative estimation of eggs, larvae and oocysts, named FLOTAC, was evaluated for suitability in the diagnosis of *A. abstrusus* infection. The authors reported that it was more sensitive than the Baerman test (Gaglio et al., 2008). However, the major limitation of FLOTAC, and copromicroscopy in general, is the impossibility of making diagnosis during 1-2 months of pre-patency when radiographic changes are already evident, or when egg shedding has stopped but parasites persist and clinical signs still occur (Losonsky et al., 1983; Payo-Puente et al., 2008; Dennler et al., 2013; Schnyder et al., 2014; Febo et al., 2019). A well-trained observer is required to differentiate the species of the strongylid L1 forms on the basis of their morphometric and

morphologic characteristics (Figs. 5 and 6; Brianti et al., 2012; Traversa and Di Cesare, 2013).

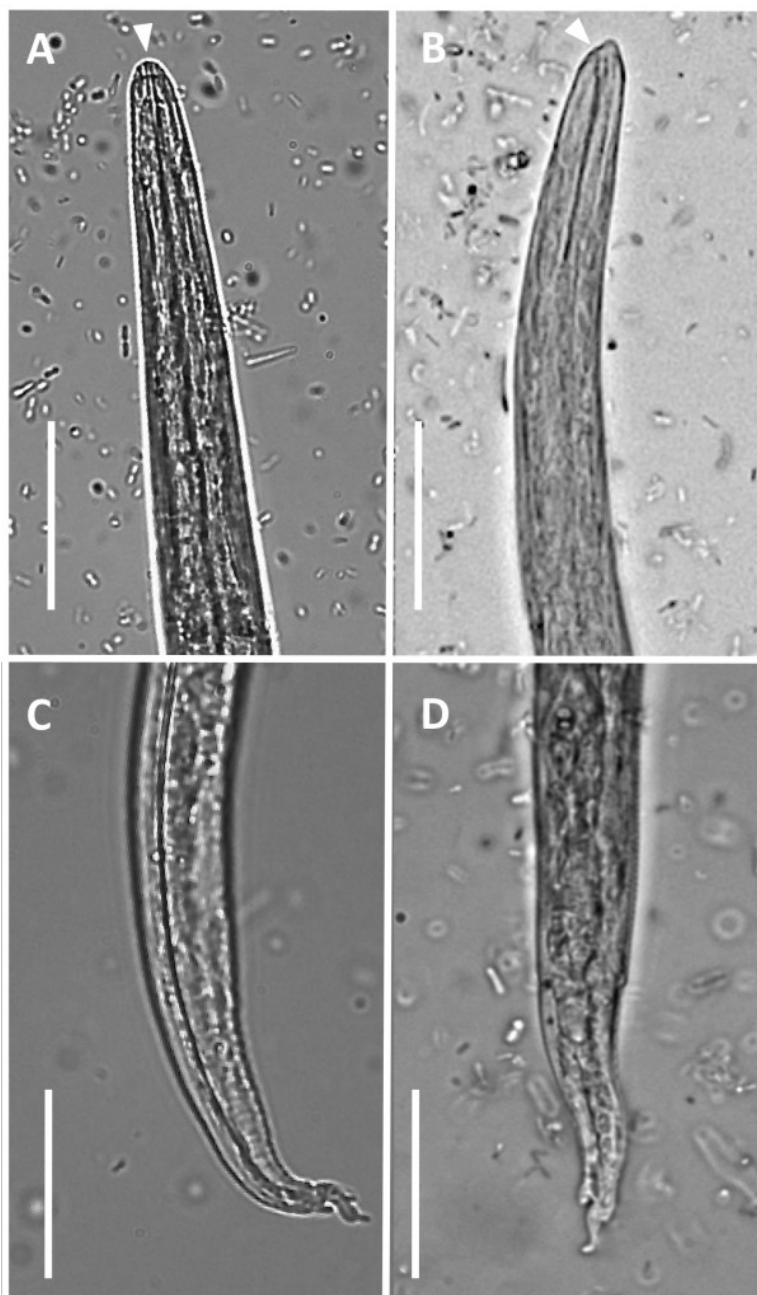


Fig. 5. Light microscopy. First stage larvae of *Aelurostrongylus abstrusus* (A-C) and *Troglostrongylus brevior* (B-D). A) Anterior extremity of *A. abstrusus*, lateral view. Note the terminal oral opening (arrowhead). B) Anterior extremity of *T. brevior*, lateral view. Note the pointed head and the sub-terminal oral opening (arrowhead). Morphology of tail of *A. abstrusus* (C) and *T. brevior* (D) showing a dorsal spine at the end of the tail. Scale bars = 25 μ m. Courtesy of E. Brianti, University of Messina, Italy

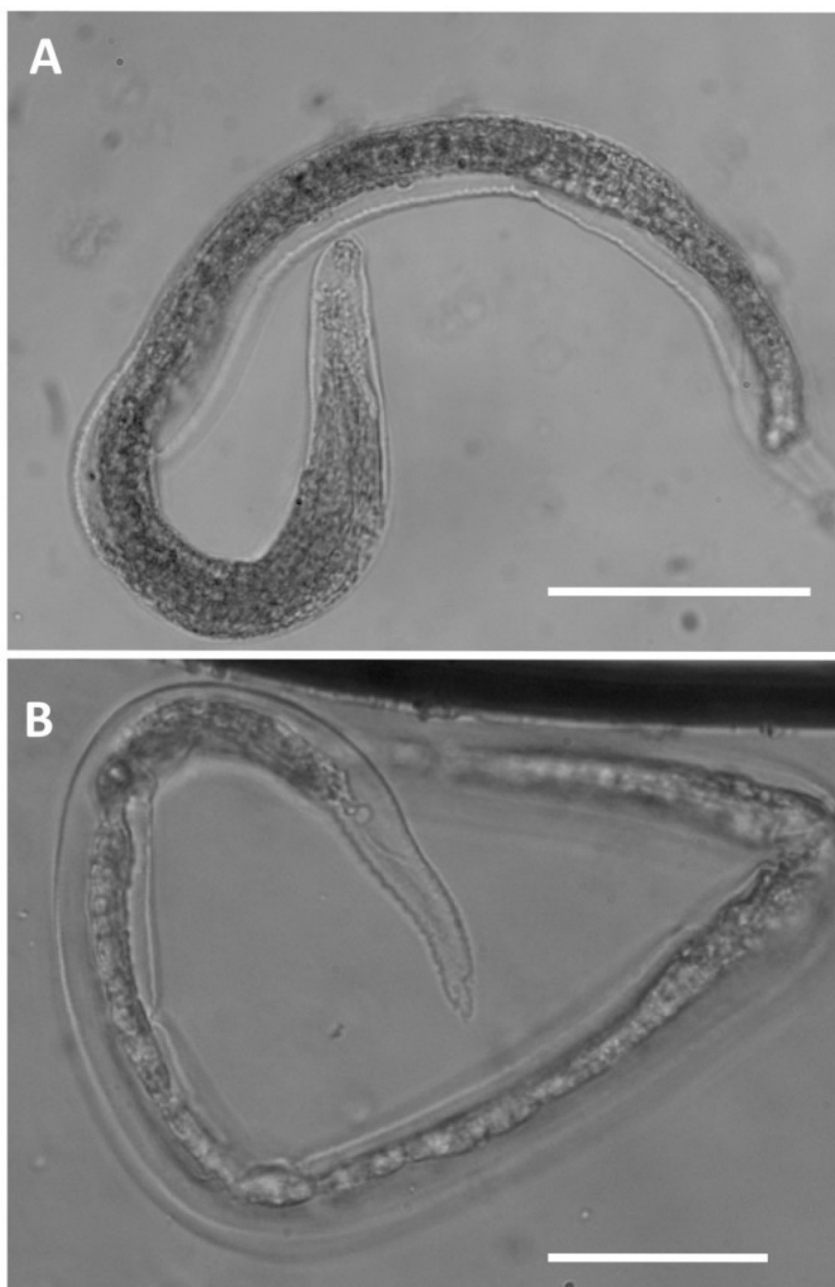


Fig. 6. Light microscopy of Oslerus rostratus first-stage larvae. Note the morphology of cephalic (A) and the caudal (B) regions. Scale bars = 100 μm. Courtesy of E. Brianti, University of Messina, Italy

Lungworm larvae can be found by cytological evaluation of tracheal swabs or washings and bronchoalveolar lavage (BAL) or lung fine-needle aspiration samples, but with less sensitivity than in faeces; so there is no benefit in performing these riskier procedures over faecal examination methods for a parasitological diagnosis, particularly in case of severe respiratory disease (Lacordia et al., 2009; Gambino et al., 2016).

Significant progress has been made in diagnosis using molecular methods. Species-specific nested-PCR assays are available for *A. abstrusus*, *T. brevior*, *C. aerophila* and *A. chabaudi*. These assays have been validated on different biological samples (faeces, floatation supernatant, Baerman sediment and pharyngeal swabs) collected from cats with natural infections. The PCR test for *A. abstrusus* provides 100 % specificity and a sensitivity of up to 96.6 %, with the best results obtained using pharyngeal swabs (Traversa et al., 2008b). New multiplex PCR assays are also available for the simultaneous detection of two or three different species including *A. abstrusus*, *T. brevior* and *A. chabaudi* (Annoscia et al., 2014). These methods allow early diagnosis in the pre-patent phase with a potential positive impact on prognosis. Molecular techniques are expected to significantly improve the understanding of the infection

biology of lungworm infections.

Antibodies to *A. abstrusus* can be detected as early as three weeks p.i. by IFAT, but past and currently active infections cannot be differentiated by serology (Hamilton and Roberts, 1968).

Angyostrongylosis was diagnosed at necropsy by the detection of adult worms in the proximal tract of the pulmonary arteries and L1 larvae were never found in domestic cat faeces (Varcasia et al., 2014b; Traversa et al., 2015).

Capillariosis is diagnosed by standard faecal flotation but molecular techniques are also available as screening tests and for investigating human cases (Traversa et al., 2010; Di Cesare et al., 2012).

Paragonomiasis is diagnosed by formalin-ether sedimentation technique (Sohn and Chai, 2005). Molecular methods are available for epidemiological purposes in cats and are used for human cases too (Intapan et al., 2005; Tantrawatpan et al., 2013).

Treatment

Information on the efficacy of various drugs is now available, not only from case reports or case series, but also from controlled studies (Table 3). However, controlled studies have often evaluated only parasitological efficacy of treatments while case reports give more information on the severity of disease of treated cats and on the clinical efficacy of therapy. The elimination of parasites is the goal of anthelmintic treatments, however, this is not enough to guarantee a favorable prognosis for severe cases of bronchopneumonia that need medical care in an intensive care unit because of respiratory failure (oxygen administration). Bacterial secondary infections usually contribute to the aggravation of the disease, thus, broad-spectrum antibiotics should always be given together with corticosteroids at anti-inflammatory doses. In the case of a pleural effusion or pneumothorax, immediate resolution by thoracocentesis is required for stabilization of the cat before other clinical diagnostic procedures are performed.

The first drug used against *A. abstrusus* was fenbendazole. Different dosages and durations of therapy (from 20 mg/kg for five days to 50 mg/kg for 15 days) were given. Furthermore, an oral paste was licenced in the UK and some other countries for treating aelurostrongylosis in cats at 50 mg/kg once a day for three days (Traversa et al., 2010). Two spot-on formulations administered at the recommended dosage were compared to a three-day-course of therapy with fenbendazole and they were found to be effective against *A. abstrusus* and safe in the treatment of twelve naturally infected cats each: one formulation containing imidacloprid 10 % and moxidectin 1 % (Advocate®, Bayer), the other emodepside 2.1 % and praziquantel 8.6 % (Profender®, Bayer) (Traversa et al., 2009b, 2009c). The moxidectin formulation gave the best results in terms of efficacy among all the three protocols, with 100 % efficacy after 30 days (Traversa et al., 2009b). The emodepside formulation was used in a one month old kitten with clinical troglostrongylosis and recently evaluated for treating 16 cats that were clinically healthy or with mild disease. Two applications, two weeks apart, were effective in clearing larvae shedding (Traversa et al., 2018, 2019b). A case series study evaluated the efficacy of combined imidacloprid 10 % and moxidectin 1 % in cats with natural *A. abstrusus* infection. Cats were rechecked on day 14 and those still found positive (4/7) were re-treated and rechecked one week later. At this time, one cat remained positive and was treated for a third time. At the end of the study (on day 50), two negative faecal tests had been obtained for all treated cats (Brianti et al., 2008). In a further study, the imidacloprid 10 % and moxidectin 1 % spot-on formulation was significantly effective against *C. aerophila* infection (Traversa et al., 2012).

A combination of milbemycin oxime (4 mg) and praziquantel (10 mg) (Milbemax®, Novartis) was administered as single oral dose (half tablet per kg) three times, 15 days apart, to a kitten affected with *A. abstrusus* bronchopneumonia and pulmonary hypertension, and this resulted in a parasitological and clinical cure (Dirven et al., 2012).

Standard topical administration of selamectin spot-on formulation (6 mg/kg) (Stronghold®, Zoetis) was used in some cases (Reinhardt et al., 2004; Grandi et al., 2005; Iannino et al., 2013). In one study, selamectin was effective in one of four cats at day 30 and in two of the three cats re-treated and followed up at day 60 (Grandi et al., 2005). In the other study, treatment was effective in nine of ten cats (Iannino et al., 2013).

The efficacy of a spot-on combination of fipronil 8.3 %, (S)-methoprene 10 %, eprinomectin 0.4 % and praziquantel 8.3 % (Broadline®, Merial) was evaluated under experimental conditions and it was found to be highly effective for both the prevention and treatment of *A. abstrusus* infection by killing both adult and immature parasites (Knaus et al., 2014). Parasitological efficacy of this spot on combination was also confirmed in natural infections from *A. abstrusus*, *T. brevior*, *O. rostratus*, *C. aerophila* or mixed infections (Giannelli et al., 2015, 2017).

Severe respiratory cases of troglostrongylosis were not cured by imidacloprid 10 % and moxidectin 1 % or fenbendazole treatments (Brianti et al., 2012). Mixed infections caused by *T. brevior* and *A. abstrusus* or *C. aerophila* were treated in two kittens using the emodepside 2.1 % and praziquantel 8.6 % spot-on combination but in one case two administrations were required to clear *Troglostrongylus* larval shedding (Di Cesare et al., 2014b).

A combination of milbemycin oxime (4 mg) and praziquantel (10 mg) was administered as single oral dose (0.5 tablet per kg) in two kittens with mixed infections caused by *A. abstrusus* and *T. brevior*. The asymptomatic kitten was cured but the sibling with severe respiratory disease died two days later (Di Cesare et al., 2014a). Similarly, in a case series study, clinical recovery and parasitological cure were obtained in all but one of 27 cats affected by aelurostrongylosis, troglstrongylosis, capillariosis, or mixed infections. Different drugs were used: moxidectin (17 cats), fenbendazole (3 cats with aelurostrongylosis), milbemycin oxime (3 cats), emodepside (2 cats), or eprinomectin (1 cat with capillariosis) (Crisi et al., 2017). The only death was in a three month old kitten with a mixed infection of *A. abstrusus* and *T. brevior* that presented with dyspnoea, anorexia and lethargy. The kitten died from acute respiratory failure one week after treatment with a combination of milbemycin oxime and praziquantel. Conversely, in this study the same treatment was effective in a cat with troglstrongylosis and in another two cat with a mixed infection.

In conclusion, various drugs are now available for treating lungworm infections but, apart from different compliance between oral drugs and spot ons, kitten age and weight, pregnancy or lactation, concurrent skin lesions or drug administration have to be considered case by case to make the appropriate choice (see Table 3).

Off-label use of ivermectin has been reported with controversial results against *A. abstrusus*, and it should not be considered because of the risk of toxicity mainly in kittens (Grandi et al., 2005).

Table 3: Drugs available for treating lungworm infections

DRUG (FORMULATION)	<i>A. ABSTRUSUS</i>	<i>T. BREVIOR</i>	<i>C. AEROPHILA</i>	<i>O. ROSTRATUS</i>	COMMENTS	REFERENCES
Fenbendazole 18.75% (oral paste)*	50 mg/kg q24h for 3 days	50 mg/kg q24h for 3 days	-----	-----	Can be administered during pregnancy. Do not use in kittens < 2 wks	Traversa et al., 2009b (CS), Brianti et al., 2012 (CR, LoE)
Imidacloprid 10% / Moxidectin 1% (spot on)^	Dose as recommended, repeat at 2-wk intervals up to three times.	Dose as recommended, repeat at 2-wk intervals up to three times.	Dose as recommended, single administration	-----	Avoid in case of skin lesions. Safety during pregnancy or lactation not known. Do not use in kittens < 9 weeks or < 1 kg BW	Traversa et al., 2009b (CS), Brianti et al., 2008 (CR), Traversa et al., 2012 (CS), Brianti et al., 2012 (CR, LoE), Crisi et al., 2017 (CR)
Emodepside 2.1% / praziquantel 8.6% (spot on)*	Dose as recommended, repeat once after 2 wks	Dose as recommended, repeat once after 2 wks	Dose as recommended, repeat once after 2 wks	-----	Avoid in case of skin lesions, or concurrent prednisone treatment. Can be administered during pregnancy and lactation. Do not use in kittens < 8 weeks or < 0.5 kg BW.	Traversa et al., 2009c (CS), Böhm et al., 2015 (CS, Ex), Di Cesare et al., 2014b (CR), DiCesare et al., 2015 (CR), Crisi et al., 2017 (CR), Traversa et al., 2019 b (CS)
Fipronil 8.3%, (S)-methoprene 10%, eprinomectin 0.4% and praziquantel 8.3% (spot on)*#	Dose as recommended	Dose as recommended	Dose as recommended	Dose as recommended	Avoid in case of skin lesions. Safety during pregnancy not known. Do not use during lactation and in kittens < 7 wks or < 0.6 kg BW	Knaus et al., 2014 (CS, Ex), Giannelli et al., 2015 (CS), Gianelli et al., 2017 (CR), Rehbein et al., 2014 (CR)

DRUG (FORMULATION)	A. <i>ABSTRUSUS</i>	T. <i>BREVIOR</i>	C. <i>AEROPHILA</i>	O. <i>ROSTRATUS</i>	COMMENTS	REFERENCES
Milbemycin oxime (4 mg) / praziquantel (10 mg) (oral tablet)	1 tablet per 2 kg repeat twice at 2-wk interval	1 tablet per 2 kg repeat twice at 2-wk interval	-----	-----	Can be administered during pregnancy and lactation. Do not use in kittens < 6 wks or 0.5 kg BW	Dirven et al., 2012 (CR), Crisi et al., 2017 (CR), Di Cesare et al., 2014a (CR, LoE)
Selamectin 6% (spot on)	Dose as recommended, repeat monthly 1-2 times	-----	-----	-----	Avoid in case of skin lesions. Can be administered during pregnancy and lactation. Do not use in kittens < 6 wks or underweight	Grandi et al., 2005 (CR), Iannino et al., 2013 (CR)
* licensed for treatment of A. <i>abstrusus</i>						CS=controlled study
# licensed for treatment of T. <i>brevior</i>						CR=case report
^ licensed for treatment of C. <i>aerophila</i>						Ex=experimental infection
						LoE=lack of efficacy

Prognosis

In cases of *A. abstrusus* or *T. brevior* infections, a delay in the diagnosis and treatment may lead to a worsening of clinical signs and risk for lethal cardio-pulmonary lesions, while early diagnosis and treatment greatly improve the prognosis. For instance, three out of four asymptomatic untreated control cats with natural troglostrongylosis in a recent study developed clinical signs during four weeks of evaluation (Traversa, 2019b). This may explain why in some cases affected cats die despite being treated with the drugs said to be effective against the causative lungworm species (Brianti et al., 2012; Crisi et al., 2017).

The lungworm species and the level of larval burden measured by the Baerman test is usually related to the severity of the disease, but prognosis should be mainly based on physical examination (severity of dyspnoea and occurrence of cyanosis) and radiographic findings (severity of diffuse bronchial, alveolar and interstitial disease). Some fatal cases may, however, have only mild thoracic radiographic abnormalities at diagnosis. Moreover, kittens affected by troglostrongylosis have a more severe prognosis (Crisi et al., 2018).

Prevention

Stray and free-roaming cats have a higher risk of becoming infected with lungworms in endemic areas (Iorio and Traversa, 2008). Avoiding predation is useful for preventing infections caused by metastrongyloid or trematode pulmonary worms with indirect life cycles and it is the only preventative measure for the latter. The spot on formulation containing eprinomectin (Knaus et al., 2014) is licenced apart from adult stages also against L3 and L4 forms of *A. abstrusus* and L4 of *T. brevior* and therefore it can prevent disease.

Zoonotic risk

Capillaria aerophila has zoonotic potential and sporadic cases of human capillariosis have been described worldwide. The disease manifests in humans as bronchitis with a productive cough, but the presence of haemoptysis and nodular infiltrative lesions in the lung requires a differential diagnosis regarding lung cancer (Lalosević et al., 2008).

Paragonimiasis is a food-borne zoonosis acquired by people eating raw crustaceans. Infected cats are not dangerous for people (Macpherson, 2005; Liu et al., 2008).

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