**Canine Angiostrongylus vasorum**

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**ABSTRACT**

The French heartworm _Angiostrongylus vasorum_ is a parasitic nematode that lives in the pulmonary vessels and the heart of canids. Transmission occurs through ingestion of infected intermediate hosts, such as snails and slugs. There are increasing reports of autochthonous infections in our neighbouring countries. Clinical signs usually relate to the respiratory system, coagulopathy and the neurologic system. Anorexia, gastrointestinal dysfunction and weight loss are also frequently observed. Diagnosis is not straightforward, but abnormalities detected by thoracic radiography, echocardiography, magnetic resonance imaging (MRI) or computed tomography (CT) scan can be helpful. Eosinophilia, regenerative anemia and thrombocytopenia with or without abnormalities in the coagulation profile can occur. Definitive diagnosis is made by demonstrating the parasite in the cerebrospinal fluid, in faeces (Baermann technique) and/or in broncho-alveolar lavage fluid. Treatment consists of anthelmintic drugs and supportive care if necessary.

**LIFE CYCLE AND EPIDEMIOLOGY**

**Life cycle**

The indirect life cycle (Figure 1) of _A. vasorum_ has an essential developmental phase in an intermediate (several species of slugs and snails) or paratenic host,
for example frogs. The term paratenic hosts is used to describe hosts which are not necessary in the life cycle of a parasite but nonetheless can serve as a source of infection for other hosts. They are also referred to as transfer or transport hosts (Wang et al., 2008) (Bolt et al., 1993). First stage larvae (L1) mature and moult in the intermediate hosts into infective L3 larvae (L3). The ingestion of infected intermediate or paratenic hosts results in the infection of the definitive host. The infection of the definitive host can also be caused by the ingestion of free-living L3 because L3 can leave the intermediate host and survive in water or on wet grass. The importance of this latter infection route has not yet been investigated in vivo (Barçante et al., 2003; Denk et al., 2009). Once inside the small intestine, the L3 penetrate the intestinal wall and migrate to the abdominal lymph nodes where the third and fourth moult takes place. L5 larvae migrate through the lymphatics to the portal and hepatic veins and reach the right ventricle and pulmonary arteries, where they develop into adult worms. Female worms produce eggs that are carried to the lung capillaries, where embryonation takes place. L1 larvae penetrate the bronchial walls and alveoli and are coughed up and swallowed by the host. They are excreted with the faeces where they can survive for some days. After a long and variable prepatent period (28-108 days), dogs can excrete larvae for several years. Oliveira-Junior et al. (2006) showed that experimentally infected dogs could excrete larvae for more than 600 days, and they concluded that dogs are an important reservoir for infection, even in an urban environment.

**Signalment**

All dog breeds and ages can be infected as long as they scavenge outside, although young dogs seem to be predisposed to clinical infection. In the ‘Copenhagen Angiostrongylus Survey’ (CAS), more than 50% of the infected dogs were younger than one year (Koch and Willlesen, 2009), and in the study of Chapman et al. (2004), the clinically affected dogs, on average, were only ten months old. The reasons for this age distribution are not completely clear, but the inquisitiveness and scavenging behavior of these young dogs may be of importance. Frequently playing with and eating snails is a known risk factor. Moreover, the immune system continues to develop until the age of one, making young animals more susceptible to clinical infection (Day, 2007).

In the Danish survey, no breed predisposition was reported, although others have found a higher occurrence in Cavalier King Charles spaniels, Staffordshire Bull terriers (Chapman et al., 2004) and beagles (Conboy et al., 2004). The last author attributed this fact to the use of this breed as a hunting dog. Dogs used for hunting seem to be more at risk because of their exposure to infection from the fox-snail life cycle during training (Conboy et al., 2004).

**Epidemiology**

Several factors are important in the epidemiology of Angiostrongylus infections, but their individual importance is not yet clear.
Geographically, an expansion of the distribution of the infection is suggested, although the majority of the information on distribution originates from case reports from Germany (Denk et al., 2009; Barutzki and Schaper, 2009), the Netherlands (Van Doorn et al., 2009), Denmark (Taubert et al., 2009), Canada (Conboy, 2004; Bourque et al., 2008) and the United Kingdom (Chapman et al., 2004, Yamakawa et al., 2009). Persistently hyperendemic foci, with a region of sporadic cases around them, are described. The scattered and local appearance of the parasite suggests a difference in the microclimate in which the intermediate host thrives to a greater or lesser extent (Morgan et al., 2009). Climatic factors are indeed important because slugs and snails thrive in wet and moderate climates. Slugs in particular are sensitive to lower temperatures; in cold conditions, they are less active and survive for less time (Morgan et al., 2009). Climate change and the resulting increase in warm, moist winters in northern areas might contribute to the spread of infection. Another possible explanation is the influence of environmental conditions on the survival of the free-living L3 larvae (Taubert et al., 2009).

For the definitive hosts, the infection prevalence is remarkably higher in foxes compared to dogs (5-56% in comparison to 1-9.8%, the higher figure in hunting dogs in Denmark) (Barutzki and Schaper, 2009; Koch and Willesen, 2009). Wild reservoir species may be responsible for an expansion of the disease, as foxes can range over large distances and are increasingly present in (peri)urban areas (Koch and Willisen, 2009).

As a final contribution, we must note the increased travelling and non-controlled transportation of dogs, as this can result in a worldwide spreading of the infection (Jeffries et al., 2010).

PATHOGENESIS AND CLINICAL SIGNS

The clinical presentation and its severity are highly variable, ranging from subclinical disease to sudden death. Respiratory symptoms are frequently reported, but neurologic signs and coagulopathy are also possible. Symptoms might already be chronic when animals are diagnosed with the infection (Chapman et al., 2004).

Dogs with respiratory disease present with a (chronic) history of gagging, coughing, exercise intolerance and tachy- or dyspnea with or without cyanosis. These symptoms are caused by an interstitial pneumonia and inflammation caused by migrating L1 or can also be due to decompensated heart failure due to pulmonary hypertension in more severe cases (Chapman et al., 2004; Nicolle et al., 2006). An acute respiratory crisis may occur and is frequently accompanied by lung bleeding or a hemotorax (Sasanelli et al., 2008).

A second manifestation of infection is bleeding abnormalities, which are mostly diagnosed in referral practices. Many types of bleeding at any possible location (petechiae and ecchymoses as well as extensive hematomas and bleedings in abdominal and thoracic cavities) may be seen and several parts of the coagulation pathway can be disturbed. Prothrombin time (PT), activated partial thromboplastin time (APPT) and D-dimer levels may be elevated, and fibrinogen levels may be low (Ramsey et al., 1996). It is assumed that the presence of parasites activates coagulation and clotting factors and that platelets are consumed. This is followed by intravascular fibrinolysis. Coagulation pathways are activated in two ways, the intrinsic pathway (by direct damage to the epithelium through the deposition of immune complexes) and the extrinsic pathway (by the release of tissue factors from damaged tissue) (Bourque et al., 2008). Likewise, Koch and Willesen (2009) described thrombocytopenia and elevated D-dimer levels in 80% of the cases, which may suggest a low-graded disseminated intravascular coagulopathy (DIC). Other explanations for thrombocytopenia are an autoimmune reaction with the formation of antiplatelet antibodies or platelet elimination by activated macrophages (Gould and McInnes, 1999). An acquired deficiency in von Willebrand factor (VWF) has been described in a case report of a dog affected by angiostrongylosis (Whitley et al., 2005). In cases of DIC in humans, a normal or even increased level of VWF has been observed. In veterinary medicine, however, information concerning the VWF levels in DIC cases is lacking. Whitley et al. (2005) proposed an accelerated removal of the circulating VWF as an explanation for the lower VWF levels. The mechanism includes specific or non-specific antibodies forming complexes with VWF and the removal by Fc-receptor bearing cells. In general, one should always consider angiostrongylosis in the differential diagnosis of bleeding problems in dogs living in endemic areas (Chapman et al., 2004; Helm et al., 2010).

Bleeding in the brain or spinal cord can cause neurologic symptoms. Craniotential bleeding can cause epileptic seizures, paresis and abnormal postural reactions (Wessman et al., 2009). In cases of cerebellar bleeding, hypermetria, vestibular symptoms and opisthotonus are observed, and if the brainstem is affected, abnormalities of the cranial nerves can be seen (Negrin et al., 2008). As in humans infected with A. cantonensis, inflammation has been postulated to be another potential cause of these neurologic signs. Hypoxia and parasitic emboli can also cause neurologic symptoms (Bourque et al., 2008).

Finally, uveitis, depression, weight loss, anorexia and occasionally vomiting and diarrhoea are reported (Koch and Willesen, 2009; Storms and Verdonck, 2011).

DIAGNOSIS

Clinical examination

Lung auscultation is usually normal, but in advanced cases, crackles (in most cases generalized) can be detected. With chronic pulmonary hypertension due to larval thrombosis, a systolic murmur can be heard over the tricuspid valve (Traversa and Guglielmini et al., 2008). The exact position of the murmur can be varia-
ble due to a shift in the location of the heart (Nicolle et al., 2006).

**Medical Imaging**

Thoracic radiographs reveal most commonly a multifocal/peripheral bronchointerstitial pattern with alveolar patches (Willesen et al., 2009). When the disease progresses, an alveolar pattern at the periphery of the lungs appears. This is likely due to the formation of granulomas and the bleeding caused by the migration of L1 larvae (Helm et al., 2010). In the more chronic stage, an interstitial pattern occurs that is caused by pulmonary consolidation and lung fibrosis. After resolution of the infection, a mild interstitial pattern can remain visible. Other abnormalities that can be seen are right-sided heart enlargement, dilatation of the truncus pulmonalis and, rarely, a rather eminent appearance of the pulmonary vessels. Pleural effusion (hemothorax) and a broadening of the mediastinum can also be present (Traversa and Guglielmini, 2008; Boag et al., 2004).

High resolution computed tomography can also contribute to a more accurate evaluation of the lung lesions. Consolidation in the periphery of lung lobes (especially the caudal lobes) and a patchy multifocal opacity can be seen. In severe cases, a general diffuse attenuation throughout the entire lung can occur due to the infiltration of inflammatory cells (Koch and Willesen, 2009; Helm et al., 2010).

In patients manifesting neurological signs, magnetic resonance imaging or myelography are useful diagnostic tools. In myelography, intramedullary contrast accumulation similar to that seen in myelomalacia has been reported (Wessmann et al., 2006). However, the most sensitive method to detect intracranial and intramedullary bleeding is MRI. The exact appearance of the bleeding depends on the chronicity of the lesion. Although in theory it should be possible to visualize the adult parasites, they have never been reported, and the chance of observing a sufficiently long section of a parasite to make identification possible, is rather small (Whitley et al., 2005).

Cardiac ultrasonography and Doppler are standard methods for the evaluation of heart morphology and function. Right atrial and right ventricular dilatations in combination with a diminution of the left ventricle size, change in the pulmonary flow profile (pulmonary arterial hypertension or PAH) and secondary regurgitation over the pulmonalis/tricuspidalis valve can be seen (Nicolle et al., 2006). The occurrence of PAH in dogs affected with angiostrongylosis is estimated to be less than 5% in first-line practice but can increase to more than 33% in referral hospitals (Koch and Willesen, 2009). The abnormalities mentioned above are not always present nor are they specific for Angiostrongylosis.

**Blood and cerebrospinal fluid (CSF) analysis**

Abnormalities on routine hematology are variable and depend on the chronicity and severity of the infection. Regenerative anemia (due to blood loss), eosinophilia, thrombocytopenia and, less frequently, leukocytosis and neutrophilia can be observed. The fact that these findings normalize after treatment supports the hypothesis that a low-graded immune reaction/response occurs in every dog infected with Angiostrongylus (Chapman et al., 2004, Willesen et al., 2009).

Blood biochemistry can reveal an increased serum total protein concentration, alkaline phosphatase (AP) activity, globulin, bilirubin, cholesterol and fructosamine concentrations (Chapman et al., 2004; Willesen et al., 2009). Other authors, however, did not find significant differences in experimentally infected dogs for serum alanine aminotransferase (ALT), gammaglutamyl transferase (GGT) and AP activities for urea and creatinine concentrations but did report an increase in α1-, α2- and β-globuline concentrations in the acute phase of infection (Cury et al., 2005). In experimental infections, serum aspartate aminotransferase (AST) concentrations increased slightly but not significantly. The rise of AST occurred simultaneously with creatine kinase isoenzyme MB (CK-MB). This enzyme is an indicator of heart damage, and its increase runs parallel to the arrival of the parasite in the heart (Cury et al., 2005).

In cases with bleeding problems, a prolongation of coagulation times with or without thrombocytopenia and other signs of DIC can be observed. Whitley et al. (2005) reported a prolongation of the buccal mucosal bleeding time (BMBT) and a decreased level of VWF. After the administration of desmopressin, the BMBT normalized, and after treatment of angiostrongylosis, VWF also normalized.

Examination of CSF is often indicated in animals showing neurological signs. An abnormally high protein content, signs of erythrophagia and high red blood cell counts in combination with normal white blood cell counts are typical findings in dogs with bleeding in the central nervous system (Wessmann et al., 2006).

**Faecal examination**

*A. vasorum* L1 larvae can be detected in faecal samples. L1 larvae (334-380 µm) are recognized by the morphology of their tails (Figure 2). They have a typical indentation of the cuticle and a bulging at the dorsal surface of the tail called a dorsal notch and a dorsal spine, respectively. A smaller ventral indentation can also be seen (Deplazes, 2006; Bourque et al., 2008; McGary and Morgan, 2009).

Faecal samples can be examined by faecal smear, a flotation technique or the Baermann technique. In urgent cases, an attempt to diagnose the disease can be made using a direct faecal smear for which a sensitivity of 54-61% has been reported (Humm and Adamantos, 2010). However, the Baermann method is considered the gold standard for the definitive diagnosis of angiostrongylosis. Nevertheless, the Baermann technique also has limitations. Fresh samples are required because the larvae need to migrate through a mesh wire. This is followed
by passive sedimentation in the Baermann funnel, which takes 24 to 36 hours and makes this test time consuming. More importantly, several authors have reported a negative Baermann test in dogs with angiostrongylosis (Denk et al., 2008). During the long pre-patent period, animals can display symptoms, but the larvae cannot yet be detected in the faeces. Moreover, the excretion of larvae is intermittent and variable. For this reason, it is advisable to collect faecal samples over a period of three consecutive days. A negative Baermann result does not exclude infection in a dog with typical symptoms living in a high-risk environment (Traversa and Guglielmini, 2008; Helm et al., 2010).

**Bronchoalveolar lavage**

Bronchoalveolar lavage (BAL) can be useful to examine cells and material from the trachea and lungs. When lesions are present in the lungs, the parasite can be demonstrated together with an increase in neutrophils, eosinophils and giant polynuclear cells (Barçante et al., 2008). The disadvantages of this technique are the potential risks, the associated morbidity (cough and crackles), the need for sedation/anesthesia and the poor sample recovery (small volumes). Furthermore, when there are no significant lesions in the lung, this test will be negative (Chapman et al., 2004).

**Serology and molecular techniques**

Serologic testing has a few inherent problems, such as cross reactivity between several endoparasites and the lack of differentiation between past and current infections. Nevertheless, there are some promising tests that have been developed, but none are commercially available. Verzberger-Epstein et al. (2008) developed a sandwich ELISA for the detection of circulating antigen in sera with a specificity of 100% (no cross reactivity with *Crenosoma vulpis*) and a sensitivity of 98% (two false negatives due to a low worm burden), which is a better result than the Baermann test. Recently, Schnyder et al. (2011) have developed a very sensitive (95.7%) and specific (94%) sandwich ELISA for the detection of circulating antigen. The authors believe that this test is a valid alternative for the diagnosis, follow-up and mass screening.

Molecular testing has great potential but unfortunately is currently only used in experimental models and is not yet commercially available (Al-Sabi et al., 2010). A real-time PCR assay has been developed which is able to amplify a region of the second internal transcribed spacer (ITS-2) of *A. vasorum* in both definitive host samples (200 µl EDTA canine blood as well as 200 mg of canine faeces) and intermediate host tissue (Jeffries et al., 2009).

**Necropsy findings**

Affected lungs show granulomatous pneumonia with suppurrative and eosinophilic inflammation with vascular changes (thrombosis and fibrosis). Adult worms are present in the pulmonary arteries and right heart and are surrounded by fibrin. The reddish adult males are 14-18 mm in length and have a small bursa, two long spiculae and a kinked tail. Females are larger (18-25 mm), with a vulvar orifice just before the anus and long greyish ovaria coiled around the red intestine. Larvae are found in the smaller vessels of the lungs and cause inflammation. This causes the formation of caseogranulomas at the periphery of the lungs and pleura (Bourque et al., 2008; Denk et al. 2008; Koch and Willesen, 2009). Aberrant migration of larvae can cause caseogranulomas in other organs, such as the kidney, brain, spleen, adrenals and tracheobronchial lymph nodes (Bourque et al., 2008). The occurrence of larvae in the urinary system, the eye, pericardium, pancreas, liver, muscle and the skin has also been reported (Perry et al., 1991; Oliveira-Junior et al., 2004). Myocarditis and glomerulonephritis due to a type II hypersensitivity reaction and aberrant larvae was the cause of death in one dog (Gould and Mc Innes, 1999). In severe cases with extensive tissue damage, smooth muscle hypertrophy and hyperplasia of the arterial tunica media can be seen, which may be consequences of pulmonary hypertension. In cases of bleeding problems, large hematomas can be found, and if neurologic signs were present, bleeding in the brain or spinal cord can be noticed (Garosi et al., 2005, Bourque et al., 2008).

**TREATMENT**

The treatment consists of two parts: anthelmintic therapy and supportive care. Supportive care depends on the severity of the symptoms and consists of cage rest and hospitalization with oxygen supplementation in severe respiratory cases. In cases with life threatening DIC or bleeding problems, transfusions with blood, fresh frozen plasma or packed red blood cells may be life saving. Bleeding problems tend to resolve 24 hours after anthelmintic treatment, but the exact mechanism of the resolution of the bleeding pro-
blems is not yet clear (Koch and Willesen, 2009). The use of other medications (e.g., corticosteroids, bronchodilators, diuretics, and ACE-inhibitors) is anecdotal and depends on the assessment of the clinician (Chapman et al., 2004). Corticosteroids can be helpful in anaphylactic reactions and immune-mediated thrombocytopenia and can reduce pulmonary inflammation and secondary fibrosis (Gould and McInnes, 1999; Koch and Willesen, 2009).

The use of various anthelmintics has been described in the literature, among which fenbendazole, milbemycin oxime and imidacloprid/moxidectin spot-on are the most frequently used (Table 1). The treatment with ivermectin and levamisole may have side effects. Anaphylactic reactions due to the rapid killing of parasites have been described after levamisole treatment (Bourque et al., 2008). Fenbendazole, milbemycin oxime and moxidectin/imidacloprid act more slowly, thereby reducing the chance of adverse reactions. There is no difference in efficacy between fenbendazole (25 mg/kg, SID for 20 days) and imidacloprid 10%/moxidectin 2.5% (single topical dose of 0.1 ml/kg). However, fenbendazole is not registered for this use in Europe (Gould and McInnes, 1999; Conboy, 2004). The treatment with ivermectin and levamisole may have side effects. Anaphylactic reactions due to the rapid killing of parasites have been described after levamisole treatment (Bourque et al., 2008). Fenbendazole, milbemycin oxime and moxidectin/imidacloprid act more slowly, thereby reducing the chance of adverse reactions. There is no difference in efficacy between fenbendazole (25 mg/kg, SID for 20 days) and imidacloprid 10%/moxidectin 2.5% (single topical dose of 0.1 ml/kg). However, fenbendazole is not registered for this use in Europe (Gould and McInnes, 1999; Conboy, 2004). Imidacloprid 10%/moxidectin 2.5% (single topical dose of 0.1 ml/kg) efficiently eliminates L4 stages and immature adults and side effects are rare (Willesen et al., 2007; Schnyder et al., 2009). Milbemycin oxime (0.5 mg/kg p.o.) is also efficient in eliminating infection when used once a week for four weeks (Conboy, 2004).

Six weeks after the treatment with moxidectin/imidacloprid and three weeks after the fenbendazole treatment, a Baermann test should be performed on a pooled sample from faeces collected on three consecutive days. The test should confirm cure and exclude the conversion to an asymptomatic carrier status because these carriers can act as a reservoir for infection (Chapman et al., 2004; Willesen et al., 2007). In endemic areas, reinfections can occur, and as a consequence, regular testing (every three to six months) is advisable (Koch and Willesen, 2009).

PROGNOSIS

The majority of the dogs infected with Angiostrongylus have an uneventful recovery, although this is dependent on the severity of the symptoms. Respiratory symptoms disappear within 1-2 weeks, but coughing can resolve earlier. In 40% of the severe cases, residual symptoms (cough and exercise intolerance) can occur after the elimination of the infection. An early diagnosis and treatment should help to avoid this. When PAH occurs, the prognosis depends on the severity of the infection (Koch and Willesen, 2009).

Dogs displaying neurologic signs have a good long-term prognosis independent of the symptoms caused by bleeding or inflammation (Garosi et al., 2005; Negrin et al., 2008).

If mortality occurs, it is usually caused by severe (non-compensated) bleeding or respiratory failure. In referral hospitals, mortality rates of 10-15% have been reported (Chapman et al., 2004; Koch and Willesen, 2009).

PROPHYLAXIS

Imidacloprid 10%/moxidectin 2.5% has been proven effective and is currently licensed for prophylactic use against Angiostrongylus (Schnyder et al., 2009; Schnyder et al., 2011). In hyperendemic regions, however, veterinarians should educate owners about the clinical symptoms and possible risks. They can advise removing their dogs' faeces and trying to avoid the intake of slugs and snails by putting the dog on a leash. The removal of snails is virtually impossible and ecologically not recommended (Helm et al., 2010).

CONCLUSION

Because of the emerging occurrence of A. vasorum in our neighboring countries, it should be included in the differential diagnosis of all cases presented with unexplained bleeding tendencies, respiratory and/or neurologic signs. Unfortunately, the diagnosis is not

Table 1. Frequently used drugs in the treatment of Angiostrongylus vasorum and their dosage (adapted from Helm et al., 2010).

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<th>Drug</th>
<th>Dose</th>
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| Fenbendazole          | 1) 20-25 mg/kg PO SID for 20 days  
                        | 2) 50 mg/kg PO SID for 5-21 days                  |
| Imidacloprid/ Moxidectin | topical 0,1 ml/kg single dose                      |
| Milbemycin oxime      | 0,5 mg/kg PO once a week during 4 weeks            |
| Levamisole            | 1) 7,5 mg/kg PO SID for 2 days  
                        | then 10 mg/kg for 2 days                           |
                        | 2) 12,5 mg/kg SC for 3 days                        |
| Ivermectin            | 200-400 µg/kg SC every 3 weeks  
                        | 2-4 treatments                                     |
straightforward, and the Baermann testing of mixed faecal samples is still the gold standard. However, once the diagnosis is established, the treatment is rather simple with commercially available anthelmintic products. Clinicians’ awareness of this illness and its various clinical signs is important because full recovery is possible if diagnosed at an early disease stage.

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