Case Report

Filaroidosis infection in an immunocompetent adult dog from France

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Summary

A dog from Paris (France) was referred with a 2-week history of dry cough, intermittent acute onset of dyspnoea, and acute abdominal pain. A generalised bronchoalveolar infiltrate with a patchy distribution was observed at chest x-rays and computed tomography (CT) scans. Negative results were obtained through several faecal examinations for cardiorespiratory nematodes by using the Baermann technique and at two blood analysis with a commercially available test for the detection of A. vasorum (the first one at the first visit and second one at the control visit, one month later). PCR methods for the identification of A. vasorum and C. vulpis were also accomplished. At the control visit, nematode L1s were found during direct microscopic examination of bronchoalveolar lavage fluid (BALF). Thus, a different antigen-based assay for the detection of A. vasorum was performed with a positive result. Moreover, based on morphology, isolated larvae were identified as Filaroides hirthi. The dog was treated with fenbendazole (50 mg/kg per os once daily) for two consecutive weeks. After five months, the dog was referred again for the intermittent acute onset of dyspnoea and was found to be still positive for F. hirthi larvae at BALF examination. A 15-day treatment regimen with fenbendazole in combination with three subcutaneous injections of ivermectin (0.4 mg/kg, once every two weeks), was then performed. No larvae were detected at two BALF microscopic examinations performed one month apart. Results from this case report underline the importance of including F. hirthi infections in the differential diagnosis of dog bronchopneumonia.

Keywords: Filaroides hirthi; canine verminous bronchopneumonia; France

Introduction

Reports of nematodes parasitizing the respiratory tract of carnivores are increasingly common in Europe (Traversa et al., 2010; Giannelli et al., 2017) and these parasites can cause severe and occasionally fatal impairment (Traversa et al., 2010). Of the nematode species affecting dogs, Angiostrongylus vasorum is the most common (Morgan & Shaw, 2010; Helm et al., 2010), whereas Crenosoma vulpis, Ostertus osleri and Filaroides hirthi show more limited geographic distribution (Traversa et al., 2010; Latrofa et al., 2015). In particular, Filaroides hirthi has been sporadically documented in dogs from Germany (Bahnemann & Bauer, 1994), Great Britain (Spencer et al., 1985), Australia (Beveridge et al., 1983), Japan (Kagei et al., 1976), United States (Rubash, 1986; Pinckney et al., 1988), Ireland (Torgerson et al., 1997), France (Bordeau & Ehm, 1992) and Spain (Caro-Vadillo et al., 2005). Unlike other dog metstrongylid nematodes, F. hirthi has a direct life cycle. Puppies are infected through ingestion of first-stage larvae (L1s) passed by the faeces of chronically infected bitches (Georgi et al., 1979). L1s rapidly make their way to the lungs via the he-
patic-portal or mesenteric lymphatic system and can survive within
the mesenteric lymph nodes for extended periods, thus exposing
the animal to auto-reinfection (Georgi et al., 1979). Once in the
respiratory apparatus, larvae moult into adults and within around
five weeks, females shed larvae that can be detected in the faeces
of the infected host (Bowman, 2000). Adults, causing severe bron-
chopneumonia, can remain hidden in lung parenchyma for long
periods. Clinical disease outcome has most often been diagnosed
in stressed young dogs, especially of toy breeds. However, se-
vere clinical presentations can also be observed in immunocom-
petent and immunocompromised adults (Caro-Vadillo et al., 2005;
Conboy, 2009). Canine F. hirthi infection is usually marked by dry
cough (Bowman, 2000) along with rapid breathing, dyspnoea, and
exercise intolerance (Rubash, 1986; Andreasen & Carmichael,
1992; Bourdeau & Ehm, 1992). The diagnosis of the infection is
based on direct detection of L1s in bronchoalveolar lavage or in
the faeces (Pinckney et al., 1988; McGarry & Morgan, 2009). How-
ever, due to intermittent faecal larval shedding and the occurrence
of auto-reinfections, the diagnosis and treatment of canine F. hirthi
infection remain challenging (Bauer & Bahnemann, 1996).

Case presentation

A seven-year old unspayed female West Highland white terrier
living in Paris (France) was admitted to the Small Animal Veteri-
nary Clinic Paris III (Paris, France) for decreased appetite, acute
abdominal pain, dry cough, and intermittent acute onset of dysp-
noea. Previous history included cranium-mandibular osteopathy at
the age of 10 months, successfully treated with corticosteroids.
The dog had been purchased at the age of three months in Ireland
and since had never travelled out of France. Physical exam-
ination revealed severe abdominal pain at the cranial abdominal
region, tachypnoea (50 breaths/min) and bradycardia (60 bpm),
associated with respiratory sinus arrhythmia. Thoracic ausculta-
tion disclosed pronounced bilateral wheezing and crackles, along
with increased breathing sounds in trachea. A complete blood
count showed moderate anaemia (Hgb 12.83 g/dl; reference in-
terval 13.2 – 19.2), moderate leucocytosis (13.7x10⁹/L; reference
interval 6 – 13) associated with eosinophilia (2.4x10⁹/L, reference
interval 0.0 – 1.2) and thrombocytopenia (20x10⁹/L; reference in-
terval 150 – 500). Blood smear examination highlighted the pres-
ence of giant platelets, while the coagulation profile was within
the normal reference range. No biochemical abnormality was found at
blood and urine analysis, and no abdominal malformations or ab-
normalities were recorded by either ultrasonography or abdominal
computed tomography (CT). Chest x-rays revealed an extensive
bronchoalveolar infiltrate with a patchy distribution (Fig. 1), what
was also confirmed by CT (Fig. 2A). Echocardiography revealed
no defects, while bronchoscopy showed tracheal and bronchial
hemorrhagic areas associated with mucosal hyperaemia. Cytolo-
gy of the broncho-alveolar lavage fluid (BALF) showed moderate
cell density, characterised by high levels of eosinophils (56 %) and
neutrophils (22 %) and few macrophages (22 %).

On the basis of these findings, the main differential diagnoses
included nematode, mycotic, allergic and inflammatory broncho-
pneumonia, as well as idiopathic pulmonary fibrosis, primary or

Fig. 1. Right lateral (on the left) and ventrodorsal (on the right) thoracic radiographs of the examined dog showing an extensive broncho-alveolar infiltrate and consolidated areas with a patchy distribution. Lesions are more severe in the right hemithorax.
metastatic pulmonary neoplasia, granulomatous pneumonia and pulmonary granulomatosis. Parasitological analysis on faecal samples collected over three consecutive days and examined as fresh smears, by the Baermann technique and by flotation test using a low-density flotation solution (specific gravity 1.2), proved negative for parasites. Negative results were also obtained after blood analysis with a commercially available blood test (Angio Detect™, IDEXX, Westbrook, USA) for the detection of A. vasorum antigen and also after faecal samples analysis with PCR for the detection of the A. vasorum and C. vulpis (performed by IDEXX Laboratories, France). Bacteriological examination of BALF revealed the presence of extra-cellular Pseudomonas species. Real-time PCR on BALF was negative for Toxoplasma gondii and Pneumocystis carinii (Biomnis laboratories, France). Idiopathic eosinophilic bronchopneumonia was suspected and the dog was treated with marbofloxacin (4 mg/kg/day, Marbocyline; Vetquinoil S.A.) during 15 days and prednisolone (10 mg/kg/day, Demipred®; Sogeval) during 30 days. Treatment resulted in moderate improvement in clinical condition and thoracic pulmonary lesions by CT examination (Fig. 2B) at control visit, performed one month later. In contrast, the BALF control cytological analysis, performed 30 days after the beginning of the treatment, revealed absolute neutrophilia (43 %) with normal eosinophilia (3 %) associated with a large number of nematode L1s found at direct microscopic examination of BALF. The suspicion that at the first visit the dog had an A. vasorum infection in the prepatent period, led us to repeat the antigen blood test (Angio Detect™, IDEXX, Westbrook, USA) and the Baermann test at the control visit. Both these tests were negative for a second time. Therefore, a different antigen-based assay (Schnyder et al., 2011) with a positive result for the detection of A. vasorum was performed. Additionally, based on morphology and dimensions according to previously reported data (McGarry & Morgan, 2009) the L1s found in BALF were microscopically identified at the species level. The collected L1s measured 265 ± 13 µm and were characterised by a straight tail with a single slight dorsal indentation, ending in a lance-like shape (Fig. 3). Larvae isolated from faecal samples and BALF were also subjected to molecular identification using primers targeting partial 12S and 18S rRNA genes (Fila_12SF: 5′-CGGGAGTAAAGTTTGGTTAAACCG-3′ and Fila_12SR: 5′-CATGACGGATGGTTGTACCAC-3′; NC18SF1: 5′-AAAGATTAAGCCATGCA-3′ and NC5BR: 5′-GCAGGTTCACCTACAGAT-3′, respectively) and run PCR protocol described elsewhere (Latrofa et al., 2015). Although both genes offer useful insights into the identification of various nematode species (Hu et al., 2004; Petterson-Kane et al., 2009; Brianti et al., 2012), the amplification turned out unsuccessful likely due to the contamination by bacterial and fungal DNA (Jefferies et al., 2010). However, based on the morphological features of the larvae and clinical signs a diagnosis of F. hirshi infection was confirmed and the dog was treated with oral fenbendazole once daily (50 mg/kg; Panacur™, MSD Animal Health Srl, France) for two consecutive weeks (Rubash, 1986). Fifteen days after the start of the treatment, the owner reported improvement in respiratory signs, and repeated BALF cytological analysis showed blood cell characteristics (neutrophils 22 %, eosinophils 0 %, macrophages 73 % associated with haemosiderophages) and confirmed the absence of L1s at direct microscopic examination. Thoracic CT showed excellent improvement of the pulmonary lesions (Fig. 2C). Based on its efficacy against the immature stages and the reduction of infection levels of other cardio-respiratory nematodes, milbemycin oxide (at 0.75 mg/kg; Trifexis®, Elanco Animal Health) was then administered once monthly per os to prevent reinfections and auto-reinfections (Conboy et al., 2013a; Böhm et al., 2014; Lebon et al., 2016).

Five months later, the dog was referred again for the intermittent acute onset of dyspnoea. Chest CT showed a relapse of alveolar
opacities with patchy distribution, and direct microscopic examination of BALF revealed once more the presence of live *F. hirthi* L1s. Thus, fenbendazole treatment (50 mg/kg per os for two weeks) combined with three subcutaneous off-label administrations of ivermectin (0.4 mg/kg, once every two weeks; Ivomec®, Merial), were performed. One month later, the thoracic CT showed normal lung patterns, and no larvae were detected at two BALF microscopic examinations performed one month apart.

**Discussion**

The genus *Filaroides* includes ovoviviparous nematodes that localise in the respiratory system of dogs and wild canids. Dog infections with *Filaroides* species are considered relatively uncommon, possibly because many infected dogs are clinically asymptomatic (Caro-Vadillo et al., 2005; Caswell and Williams, 2007). Indeed, clinical diseases caused by *F. hirthi* have been generally associated with immunocompromised, stressed or young dogs (Caro-Vadillo et al., 2005). Since the literature contains only case reports, the prevalence of *F. hirthi* infection in Europe and in other geographical areas is unknown (Bauer & Bahnemann, 1996; Caro-Vadillo et al., 2005). Besides other than the frequent chronic and sub-clinical infections, this could be due to diagnostic difficulties resulting from the intermittent shedding of *F. hirthi* larvae in the faeces of infected animals. This is probably also caused by wrong identification of *F. hirthi* larvae that can be confused with the most common *A. vasorum* species. Since the larvae of *Filaroides* spp. larvae are lethargic and therefore do not migrate out of the faeces easily (Traversa et al., 2010) their detection in faecal samples examined by the Baermann method may be unlikely. However, the geographical distribution of *F. hirthi* could be truly limited, resulting in sporadic infections throughout the world. In the dog examined herein, *F. hirthi* L1s were identified at BALF microscopic examination. Based on their significantly smaller size and on their tails showing a notch followed by a constriction and a terminal lance-like end, without any kink, undulation or spine, isolated larvae were distinguished from those of *A. vasorum* showing a prominent dorsal spine and a double cuticle indentation at the caudal end (McGarry & Morgan, 2009; Traversa et al., 2010; Taylor et al., 2007). The different morphology of the caudal end also allowed their differentiation from L1s of *C. vulpis*, showing a straight and uniformly pointed tail (McGarry & Morgan, 2009; Traversa et al., 2010). The L1s of *F. hirthi*, *Filaroides milksi* and *Oslerus* (*Filaroides* osleri) are morphological identical and cannot be differentiated from each other (Traversa et al., 2010; Conboy, 2009). However, in the dog examined, characteristic *O. osleri* tracheobronchial nodules were not evidenced by bronchoscopy. On the other hand, dog *F. milksi* infection has been rarely reported in Europe (Cremers et al., 1978). Moreover, the validity of *F. milksi* and *F. hirthi* as two separate species has been questioned (Conboy, 2009).

Canine *F. hirthi* infection is often subclinical in healthy and immunocompetent dogs. Nevertheless fatal respiratory disease outcome has been reported after corticosteroid treatments or because of other immunosuppressive conditions, including chronic stress,

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Fig. 3. *Filaroides hirthi* first stage larva detected at the microscopic examination of the BALF (40x magnification). Note the straight tail with a single slight dorsal indentation (thick arrow), ending into a lance-like shape (thin arrow), consistent with *F. hirthi*. 

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generalized demodicosis or adrenal cortical carcinoma (Bauer & Bahnemann, 1996). In aged dogs, the infection can begin as non-productive cough, sometimes associated with poor general condition and acute or progressive dyspnoea or tachypnoea (Bowman, 2000; Torgerson et al., 1997).

Similarly to A. vasorum infection (Martin et al., 1993), the abdominal pain observed in the dog herein examined could be related to L1s migration through the mesenteric lymph nodes, liver or kidney or as a result of pleural or diaphragmatic inflammation. Moreover, a mixed pulmonary pattern affecting all the lung lobes, mainly characterized by broncholiths, peribronchitis and perivasculitis, focal or interstitial pneumonia, granulomatous lesions and pleural fibrosis with a predominance of interstitial and alveolar infiltration, is the most common radiographic finding for F. hirthi infections (Bowman, 2000). As described in the case here reported, free larvae in the alveolar lumen may induce an inflammatory reaction characterized by numerous neutrophilic granulocytes, while eosinophilia can be observed in peripheral blood (Bahnemann & Bauer, 1994). Negative faecal examinations results do not exclude infection by Filaroides species (Caro-Vadillo et al., 2005), where bronchial or tracheal washing are more reliable than coprology in the detection of L1s (Brownlie, 1990). Thus, F. hirthi larvae are detected most accurately by the examination of bronchial mucus (Conboy, 2009). In the present case, the Baermann method was used in association with BALF direct microscopic examination, as well as serological and molecular detection methods in order to rule out A. vasorum and C. vulpis infection.

The prevalence of A. vasorum is high in France and this cardio-respiratory nematode should be always considered in the differential diagnosis of dog bronchopneumonia. Although for the positive commercial A. vasorum sandwich-ELISA used herein a specificity of 94% has been reported (Verzberger-Epshtein et al., 2008; Schnyder et al., 2011), a possible cross-reactivity with F. hirthi has not previously been assessed. Based on the results obtained, the cross-reactivity of this immunological A. vasorum diagnostic test with F. hirthi should not be ruled out. Since this test can reveal the presence of A. vasorum antigens until 34 days after the treatment and it is always positive in dogs harbouring only one worm (Schnyder et al., 2011) the occurrence of a previous A. vasorum infection in dog examined in this study cannot be excluded. Considering the high prevalence of A. vasorum infection in France and in other European countries (Lebon et al., 2016; Lemperereur et al., 2016; Traversa & Guglielmini, 2008), great attention is thus required for interpretation of results and diagnostic procedures.

For the treatment of dog Filaroides infections, the effective use of fenbendazole (50 mg/kg oral once a day for 10 to 14 days), albendazole (25 to 50 mg/kg twice a day for five consecutive days repeated two weeks later), and single administration of injectable ivermectin at 0.4 – 1 mg/kg, has been reported in previous studies (Bauer & Bahnemann, 1996; Bowman, 2000; Caro-Vadillo et al., 2005). The treatment of F. hirthi is particularly challenging because this parasite does not require an intermediate host for its development, and reinfections and auto-reinfections frequently occur (Georgi et al., 1979; Torgerson et al., 1997). This was the main reason why milbemycin oxime and ivermectin treatments were performed in this case report. However, the treatment with oral milbemycin oxime was unsuccessful from preventing the F. hirthi infection. The efficacy of milbemycin oxime for the prevention of A. vasorum and the reduction of A. vasorum and C. vulpis infection levels, have been evidenced (Conboy et al., 2013; Böhm et al., 2014; Lebon et al., 2016). However, dosing intervals for the treatment of infections and the prevention in clinical disease have not yet been established (Conboy et al., 2013a). Moreover, for the effective treatment of other respiratory nematodes, as E. boehmi infection, milbemycin oxime should be used at the increased dose of 2 mg/kg (Conboy et al., 2013b; Cervone et al., 2017). All these factors might represent possible reasons for the failure of milbemycin oxime in the case study reported here. Though moxidectin larvicidal and adulticidal activity against A. vasorum in dogs has been demonstrated (Willesen et al., 2007; Schnyder et al., 2009), in France moxidectin is available only as a topic-spot-on formulation for its use in companion animals. Since the patient examined here had previously showed a cutaneous reaction to spot-on formulations, the prophylactic use of oral milbemycin oxime was preferred to moxidectin in this case report. Although the use of ivermectin should be discouraged in canine medicine, unless mandatory due to the lack of other efficacious drugs, injectable ivermectin was preferred in this study because of previous reports on its effectiveness for the treatment of F. hirthi in dogs (Erb & Georgi, 1982; Pinckney et al., 1988; Bauer & Bahnemann, 1996).

Based on the resolution of respiratory signs and the absence of L1s at two BALF microscopical examinations performed one month apart following the combined fenbendazole and ivermectin treatment, it can be assumed that the dog from the case here presented was healed from F. hirthi infection. However, due to lack of follow-up of the dog examined in this case report, further reinfections after this combined treatment cannot be ruled out.

In conclusion, results from this study underline the importance of including F. hirthi infections in the differential diagnosis of dog bronchopneumonia.

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