First autochtonous infection of a dog with *Oslerus (Filaroides) osleri* in the Czech Republic

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Summary

This case report describes an infection with *O. osleri* in a 10-month-old intact female Miniature German Spitz that presented with a 3-month history of progressive cough. Diagnosis was based upon visualization of characteristic lesions during bronchoscopy. Female parasites and first-stage larvae collected from tracheal nodules were morphologically identical to the larvae of *O. osleri*. First-stage larvae isolated from faeces were used for morphological and molecular confirmation of the diagnosis. Anthelmintic therapy with fenbendazole (50 mg/kg orally once daily for 2 weeks) was successful. This is the first report of autochthonous infection of a dog with *O. osleri* in the Czech Republic. Oslerosis should be considered in the differential diagnosis in young dogs with persistent respiratory signs.

Keywords: *Oslerus osleri,* dog, kennel cough, anthelmintics, parasitic tracheobronchitis, endoscopy

Erste autochthone Infektion eines Hundes mit *Oslerus (Filaroides) osleri* in der Tschechischen Republik

In diesem Fallbericht wird eine Infektion mit *O. osleri* bei einer 10 Monate alten Spitz-Hündin mit zunehmendem Husten während der letzten 3 Monate beschrieben. Die Diagnose erfolgte anhand von typischen Läsionen nach einer Bronchoskopie. Weibliche Parasiten und Larven im 1. Stadium aus Knötchen in der Trachea waren morphologisch identisch mit *O. osleri* Larven. Aus dem Kot isolierte 1. Stadium-Larven wurden zur morphologischen und molekularen Bestätigung der Diagnose herangezogen. Eine anthelmintische Behandlung mit Fenbendazol (50 mg/Kg per os, einmal täglich während 2 Wochen) zeigte sich als erfolgreich. Dies ist der erste Bericht einer autochthonen Infektion eines Hundes mit *O. osleri* in der Tschechischen Republik. Bei jungen Hunden mit Atemwegsbeschwerden muss eine Oslerosis differentialdiagnostisch in Betracht gezogen werden.

Schlüsselwörter: *Oslerus osleri,* Hund, Zwingerhusten, Anthelmintika, parasitäre Tracheobronchitis, Endoskopie

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Introduction

Nematodes affecting the cardiopulmonary system of dogs have recently become the focus of increased attention in the scientific community due to their spread into previously non-endemic regions. The growing concern of veterinarians and parasitologists is supported by an increased number of cases and increasing prevalence. This is especially true for the metastrongyloids *Angiostrongylus vasorum* and *Crenosoma vulpis*, the trichuroid *Eucoleus aerophilus* (syn. *Capillaria aerophila*) and the filaroid *Dirofilaria immitis* (Traversa et al., 2010). While bronchopulmonary parasites are a relatively rare cause of pulmonary disease in dogs, *O. osleri* has been reported more frequently (Brownlie, 1990; Schuster and Hamann, 1993; Bourdoiseau et al., 1994; Kresken et al., 1996; Saari et al., 1997; Floto et al., 2005; McGarry and Morgan, 2009; Reagan and Arohnson, 2012).

O. osleri (Cobbold, 1879) has a direct life cycle. Adults reside in nodules that are usually centered at the tracheobronchial bifurcation. The gravid female nematodes

protrude their caudal extremities through the respiratory epithelium. They deposit thin-shelled eggs containing first-stage larvae into the tracheal lumen. Eggs hatch before they are voided in the host's faeces. The first-stage larvae are directly infective. They are coughed up and either expectorated in sputum or swallowed and voided in faeces. Susceptible hosts are infected by ingestion of infective first-stage larvae. Following ingestion, the larvae penetrate the mucosa of the gastrointestinal tract, and travel to the right side of the heart via lymphatics or the hepatic venous circulation. Larvae then migrate to the lungs via pulmonary arteries. From the pulmonary capillaries, the L1 invade the pulmonary parenchyma. All 5 larval stages develop in the lung and migrate up the trachea as adults (Yao et al., 2011; Reagan and Arohnson, 2012; Bowman, 2014). Tracheobronchial nodules are formed, where the life cycle is completed. The prepatent period ranges from 10 to 21 weeks with immature worms arriving in the trachea approximately 2 months after exposure (Clayton and Lindsay, 1979, Conboy, 2009; Sherding, 2009; Yao et al., 2011; Reagan and Arohnson, 2012).

O. osleri is a parasite found in the trachea and bronchi of dogs, as well as wild canid species, such as dingoes (Dunsmorre and Spratt, 1979), coyotes (Thornton et al., 1974; Carlson and Nielsen, 1985), wolves (Dias et al., 2012; Verocai et al., 2013) and foxes (Delić et al., 1966; Dunsmorre and Spratt, 1979; Avelar et al., 2013), and has a worldwide distribution. However, wild canids do not seem to serve as an infection reservoir for dogs, as dogs exposed to infective larvae derived from coyotes failed to develop *O. osleri* infections (Foreyt and Foreyt, 1981).

The clinical signs of canine oslerosis are characterized by non-productive cough, which can be exacerbated by exercise, excitement, or tracheal palpation. Dyspnoea, exercise intolerance, cyanosis and acute respiratory distress may occur (Conboy, 2009; Reagan and Arohnson, 2012). A single case of pneumothorax due to *Filaroides (Oslerus) osleri* infection has been reported (Burrows et al., 1972). The potential for missing lungworm cases exists due to the diagnostic challenge involved in the detection of most of these parasites (Conboy, 2009). This paper describes a case of respiratory disease due to *Oslerus osleri* in a dog and its response to treatment with fenbendazole following correct diagnosis, including molecular analysis.

Case history and clinical examination

In January 2011, a 10-month-old intact female Miniature German Spitz was presented to a primary care clinic for persistent respiratory signs of approximately 3 month duration. The owner described that cough was worsened by excitement or exercise and occasional episodes of dyspnoea were observed. The dog was treated with various antibiotics (quinolones, amoxicillin with clavulanic acid and doxycycline respectively) with temporary improvement. A thoracic radiograph (lateral view) and an endoscopic examination of respiratory tract performed at another veterinary clinic had not revealed any significant findings. During physical examination the cough was easily elicited upon tracheal palpation and increased expiratory effort and abdominal breathing were noted. Thoracic auscultation revealed increased bronchial sounds.

Further examinations

Results of haematology and biochemistry were within reference ranges. Left and right lateral and ventrodorsal thoracic radiographs showed increased opacity of tracheal lining, while the pulmonary parenchyma was normal. Bronchoscopic examination revealed oedematous tracheal and bronchial walls. Near the tracheal bifurcation, multiple nodules (Fig. 1) of different sizes (2 to 6 mm in diameter) were found. The biggest nodule almost completely filled the tracheal lumen. The nodules contained nematodes, some of which protruded through the epithelium (Fig. 2). Several endoscopic biopsies of the nodules were taken and submitted for histopathologic examination. The fixed specimens were processed through the conventional paraffin embedding technique, sectioned at 5 µm and stained with hematoxylin and eosin. Histological examination revealed diffuse irregular thickening of the mucosa due to intensive oedema and moderate granulomatous inflammatory reaction around metazoan parasites embedded in the tracheal submucosa. These parasites were observed in groups and had typical nematode characteristics including the presence of body cavity, cuticule and coelomyrian muscles (Yao et al., 2011).

Parasitological examination

Morphological examinations of the nematodes (3 intact female specimens and approximately 30 first-stages larvae) recovered from two nodules were performed. Females ranged in size between 8 to 12 mm in length. They were further characterized by having a rounded anterior edge with a retracted oral opening with flask-shaped oesophagus measuring between 230 to 255 µm (Fig. 3a). The intestine was dark brown and tubular, typically running straight through the body and coiled around the uterus containing larvae. The vulva opening was in the posterior end near the anus (Fig. 3b) and fully developed larvae, surrounded by a thin shell, were

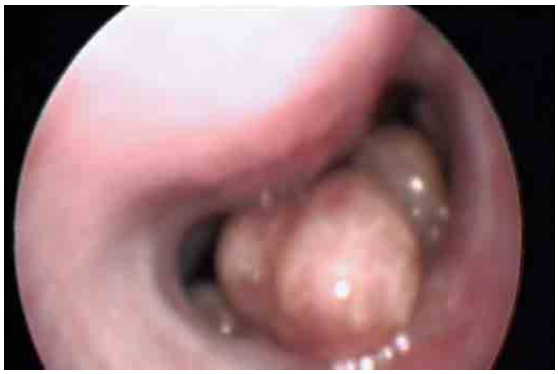

Figure 1: View of the nodules within the lumen of the trachea as seen on endoscopic evaluation.

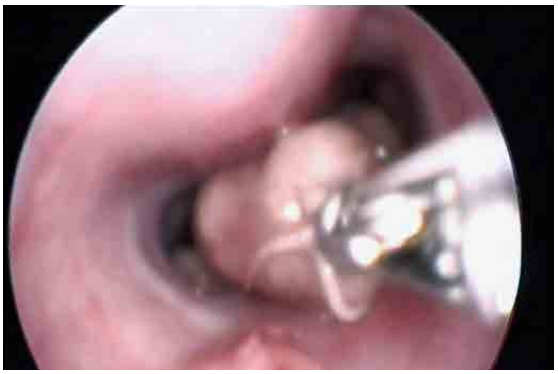

Figure 2: Nematodes protruding from tracheal nodules.

observed in the distal portion of the uterus and in the vagina. Newly hatched larvae isolated from nodules were between 220 and 250 µm long and between 20 and 24 µm wide, with a rounded head and central oral opening (Fig. 3c). Their tails (32–35 µm in length) had a distinct indentation bearing a characteristic sinus wave-shaped kink (Fig.3d). Morphology and measurements of the adult females and of their larvae were all consistent with those of *O. osleri*, as reported by Muñoz et al. (2007), Conboy (2009) and McGarry and Morgan (2009). However, slight variations were observed in the morphology of the tails.

The faecal sample was examined by modified Sheather's solution (d = 1.27 g/ml) flotation technique. Neither coccidian cysts, helminth eggs nor helminth larvae were detected. Three grams of faecal sample were used for the detection of lungworm larvae by the Baermann's larvoscopy method. First-stage larvae measuring between 315 and 360 µm were identified. They were often coiled or arch-shaped and the morphology of the tails was identical with the tails of the newly hatched larvae isolated from the nodules (Conboy, 2009; McGarry and Morgan, 2009; Traversa et al., 2010)

Parasites obtained from larvoscopy were washed three times with phosphate buffered saline (PBS) for 5 min at 3,500 × g. The supernatant was removed and the DNA isolation from the sediment performed using the NucleoSpin® Tissue kit (Macherey–Nagel, Düren, Germany) according to the manufacturer's protocol. PCR was performed using primers 391 forward and 501 reverse (Nadler et al., 2000) amplifying a 950 bp fragment of the 5' end of 28S or LSU (large ribosomal subunit) rDNA. PCR reaction of 25 µl consisted of 0.5 µM of each primer, 12.5 µl of Combi PPP Master Mix (Top Bio, Praha, Czech Republic), 8.5 µl of PCR H2O and 2 µl of DNA. The cycling parameters consisted of 35 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 1 min, plus a final extension at 72 °C for 7 min. A negative (water) control was included for PCR. PCR products were subjected to electrophoresis through a 1.5% agarose gel and stained with GoodView (Ecoli, Bratislava, Slovak Republic). The DNA from the PCR product was extracted using a QIAquick Gel Extraction Kit (Qiagen, Prague, Czech Republic) and sent for sequencing (Macrogen, Seoul, Korea). The sequence was aligned using ChromasPro and compared with sequences in GenBank. The alignment of the obtained sequence, with reference LSU partial sequence, showed 99% homology with the GenBank-listed species *Oslerus osleri* (AY292800.1). The obtained sequence has been deposited in GenBank: accession number JX185314 for LSU rDNA (5' end).

Blood samples were collected from the aforementioned dog, as well as from his dam and a littermate. Blood samples were centrifuged and serum obtained. All sera were tested with the Angio Detect™ Test (IDEXX Lab-

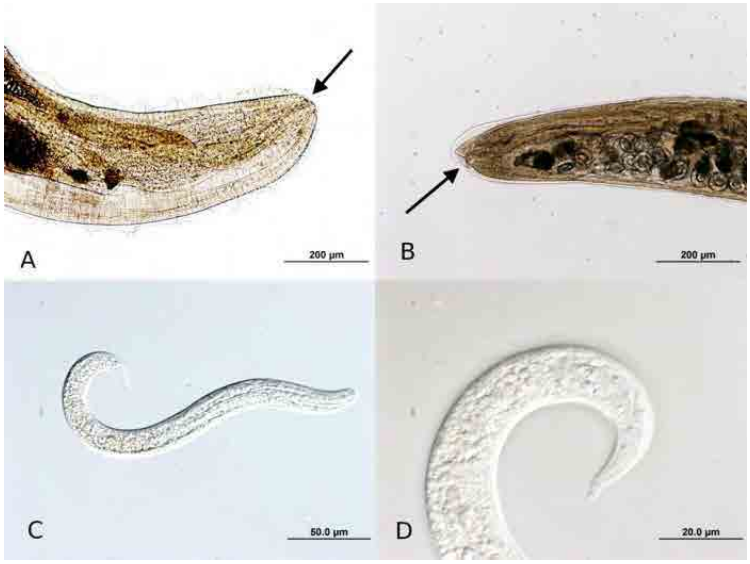

Figure 3: Light microscopy of *Oslerus osleri*. (a) Cephalic region of a female parasite, lateral view; note the retracted position of the oral opening (*arrow*). (b) Posterior end of a female nematode, lateral view; note the terminal position of the vulva near the anus (*arrow*). (c) Newly hatched larva recovered from bronchial nodule. (d) Morphology of the tail of the first stage larva with prominent indentation and distinctly wavy appearance.

oratories), a lateral flow immunochromatography test, and with an ELISA for detection of circulating antigen of *A. vasorum*. Tests were performed by experienced laboratory staff following the manufacturer's direction and within the indicated expiry dates (Schnyder et al., 2011, Schnyder et al., 2014).

Follow-up

The infected dog was treated with fenbendazole (50 mg/kg orally once daily, for 2 weeks, Panacur, Intervet, Boxmeer, the Netherlands) administered in combination with amoxicillin–clavulanic acid (20 mg/kg orally twice daily, for 2 weeks, Synulox, Pfizer, Prague, Czech Republic). The clinical signs resolved in one week. A coprological examination performed 3 weeks later was negative. Six months after treatment the owner reported that the patient remained free of clinical signs. Serum samples collected from the dog with the clinical signs, as well as from his dam and a littermate tested negative for antigen of *A. vasorum*.

Discussion

This is the first report of an autochthonous infection with *O. osleri* in a dog in the Czech Republic. Parasitic infections of the respiratory tract in dogs are relatively uncommon; young animals (< 2 years) are more susceptible than older dogs (Sherding, 2009; Yao et al., 2011). Many lungworm infections are subclinical, however, when clinical signs occur, chronic persistent cough is typical (Sherding, 2009). The reported dog showed coughing and dyspnoea. The cough was easily elicited upon tracheal palpation, which may have been due to tracheal inflammation. Common tracheal abnormalities include canine infectious tracheobronchitis (kennel cough), tracheal collapse, foreign body, tracheal stricture, hypoplasia, neoplasia, trauma, and parasites. Infectious tracheobronchitis is the most common tracheal disease. A dry, hacking paroxysmal cough is the most consistent sign. *O. osleri* infection may be easily misdiagnosed as kennel cough, especially when the dog is presented for the first time with dry cough. Kennel cough most commonly occurs where groups of dogs of different ages and susceptibility are congregated (e.g. kennel, hospital, dog show). *O. osleri* infection can be seen in individual situations, but is more often a kennel–related problem. Infection is facilitated due to the lack of an intermediate host and the presence of infectious L1 in salivary and airway secretions (Ettinger, 2010).

Results of complete blood count are non–specific, although eosinophilia may occur in some cases (Sherding 2009). In the present case, the results were within reference ranges.

The radiographic examination is helpful, particularly if the disease process is extensive and the nodules are large (Brownlie, 1990; Ettinger, 2010). The tracheal lining may be diffusely thickened, interrupted with indistinct solid masses, or show ill-defined 2 to 10 mm semicircular lesions protruding into the lumen (Brownlie, 1990; Ettinger, 2010). In the reported patient, increased opacity of the tracheal lining was noticed. The most reliable diagnostic method is bronchoscopic examination with direct visualization of the tracheal nodules (Clayton and Lindsay, 1979; Kelly and Mason, 1985; Barr et al., 1986; Bourdoiseau et al., 1994).

The definitive diagnosis is based on histological and parasitological examination of the nodules (Sherding, 2009). In the case reported here, histological examination revealed metazoan parasites with typical nematode characteristics embedded in a granulomatous inflammatory reaction. Similar findings were also described in other recent reports of canine oslerosis (Ruggiero et al., 2011; Yao et al., 2011; Reagan and Arohnson, 2012).

Morphological examinations of intact female nematodes and first-stage larvae isolated from the nodules revealed morphometric characteristics typical for the females and first stage larvae of *O. osleri* in wild canids (Avelar et al., 2013; Verocai et al., 2013) and domestic dogs (Bourdoiseau et al., 1994; Muñoz et al., 2007). In this case, *O. osleri* first-stage larvae were detected in the Baermannised faecal sample only. Modified Sheather's solution flotation technique yielded a negative result. Although false negative results commonly occur, the most reliable fecal examination is floatation with zinc sulfate solution and the Baermann examination is usually an insensitive detection method for first stage larvae of *O. osleri* due to their lethargy and reluctance to migrate from faeces (Conboy, 2009; Traversa et al., 2010). An alternative to the usually performed Baermann method for larval lungworm detection is the FLOTAC technique (Rinaldi et al., 2007; Conboy, 2009).

The characteristics of the tails of first stage larvae and their lengths appeared to be reliable for distinguishing between that of other larvae of cardiopulmonary nematodes (McGarry and Morgan, 2009; Traversa et al., 2010). However, morphological identification relies on the quantity and quality of the larval specimens and the internal structure and the distinctive morphological features of the tail of the larvae are usually not well-preserved in faecal samples (McGarry and Morgan, 2009).

The alignment of the obtained sequence, with reference LSU partial sequence, showed that *O. osleri* 28S riboso-

mal sequence from our study was 99% identical to the partial sequence of 28S ribosomal RNA gene of *O. osleri* identified in a coyote from California (Carreno and Nadler, 2003). The taxonomic validity of *Oslerus* has been widely debated in the past, and many authors have synonymized it with *Filaroides*. However, Anderson (2000) supported the validity of *Oslerus* by distinguishing the subterminal or terminal position of the female anus and vulva in *Oslerus* spp. from *Filaroides* spp., in which the anus and vulva are more distant from the caudal extremity. The morphology of *O. osleri* has never been described in detail. As with other filaroid species, detailed study and redescription may reveal many new characteristics that may be informative for phylogenetic analysis (Carreno and Nadler, 2003).

Canine infections with *O. osleri* have been treated with a variety of anthelmintics (Conboy, 2009). Administration of fenbendazole at a dose of 50 mg/kg orally once daily is recommended. Therapy should be extended to 14–21 days (Sherding, 2009; Ruggiero et al., 2011; Yao et al., 2011, Reagan and Arohnson, 2012). Because the parasite-containing nodules may interfere with physical respiratory defences (e.g., the mucociliary escalator), a secondary bacterial infection may occur (Ettinger, 2010). Surgical removal of large obstructing nodules may be required in rare situations (Ettinger, 2010). In the reported patient, the owner declined follow-up diagnostics but the absence of clinical signs suggests complete resolution of the infection.

Transmission to pups through maternal grooming is assumed to be the major transmission route in domestic dogs. Parental food regurgitation appeared to be the major means of infection in free-ranging canids (Clayton and Lindsay, 1979; Bowman, 2009). The source of infection in this case is unknown. The dam (age 4 years) of the patient was imported from Germany at an early age, while her dam (age 8 years) was imported from Canada. In total, the patient had contact with 7 dogs, all of which were clinically normal with a negative faecal examination, which is usually insensitive for the detection of *O. osleri* infection.

Conclusion

In conclusion, the presented data indicate that canine oslerosis should be considered as a differential diagnosis in dogs presenting with cough and respiratory distress. Although helminths of the canine respiratory tract are uncommon, clinicians need to consider this differential diagnosis especially in young dogs with corresponding clinical signs, and those who have failed medical management for suspected canine infectious tracheobronchitis.

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