



## Short communication

## *Aelurostrongylus abstrusus* and *Troglostrongylus* sp. (Nematoda: Metastrongyloidea) infections in cats inhabiting Ibiza, Spain

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## ABSTRACT

Multiple species of metastrongylid lungworm (Nematoda: Metastrongyloidea) have been reported to infect members of the Felidae. This study describes two metastrongylid species infecting cats in Ibiza, Spain, including clinical features of infection and diagnosis via morphological and molecular characterisation of larval stages. Cats ( $n = 7$ ) presented with suspect lungworm infection, exhibiting coughing and other respiratory signs of infection. Faecal samples were collected from each cat and were subjected to the Baermann method for the detection of first stage larvae. In four cats, two different species of larvae were observed on the basis of morphology and were further molecularly characterised by PCR and sequencing of the 18S rRNA gene. Sequence data confirmed the presence of *Aelurostrongylus abstrusus* and an unknown species of *Troglostrongylus*. Molecular characterisation of *Oslerus rostratus* is also reported for the first time. Given the diversity of metastrongylid species capable of infecting cats, and morphological similarity of larval stages, an emphasis should be placed on the use of molecular characterisation for accurate diagnosis of infection.

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### 1. Introduction

A diverse range of species of the Family Metastrongyloidea are known to infect the lungs of domestic and sylvan members of the Felidae. In domestic cats (*Felis silvestris catus*), *Aelurostrongylus abstrusus* and *Troglostrongylus subcrenatus* are reported to infect the respiratory parenchyma and *Oslerus rostratus* is known to infect the bronchial submucosa (Bowman, 2000). *Troglostrongylus wilsoni*, *Troglostrongylus brevoir*, *Skrjabinocaulus* spp. and *Gurlitia* spp., have also been reported in wild feline species (Gerichter, 1949; Sarmiento and Stough, 1956; Anderson, 1978). The lifecycle characteristics for many of these species are incomplete, however it is speculated that each utilizes gastropod intermediate hosts (Bowman, 2000).

Lizards, frogs, birds and small mammals may act as paratenic hosts for many metastrongylid species and are more likely to be predated upon by cats.

Within Europe, infections with *A. abstrusus* are well documented in domestic cats (Grandi et al., 2005; Taubert et al., 2009). Reported prevalence in southern Mediterranean countries varies from 1% in Spain (Miro et al., 2004) to 17.4% in cats in north-west Portugal (Payo-Puente et al., 2008). *O. rostratus* has also been reported in Spain (Juste et al., 1992; Millan and Casanova, 2009) with a prevalence of up to 24%. Limited data is available on the diversity and potential for co-infections of cat metastrongylid species in Europe and is virtually unknown for many of the Balearic Islands of Spain.

This study documents the clinical features of seven cats with suspected lungworm infection in Ibiza, Spain and the use of morphological and genetic characterisation of larval stages as an accurate means of diagnosing metastrongylid infection. Genetic characterisation of *O. rostratus* is also described.

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**Table 1**  
Clinical and parasitological features for cats from Ibiza with suspected lungworm infection.

Cat	Location	Age	Sex	Clinical signs	Lung X-ray	Baermann's	18S rRNA gene
1	Santa Eulalia	1.5 years	M	Coughing	Mild bronchial pattern	L1	<i>Troglostrongylus</i> sp.
2	Santa Eulalia	7 months	M	Coughing	n/a	Negative	n/a
3	San Antonio	6 months	F	Coughing	n/a	L1	<i>Troglostrongylus</i> sp./ <i>Aelurostrongylus abstrusus</i>
4	San Antonio	6 months	F	Coughing	n/a	L1	<i>Aelurostrongylus abstrusus</i>
5	San Antonio	5 years	F	Cough, laboured breathing, severe cachexia	Moderate to severe bronchointerstitial pattern	L1	<i>Aelurostrongylus abstrusus</i>
6	Es Cubells	3 months	M	Occasional cough	Mild bronchial pattern	Negative	n/a
7	Ses Salines	3 months	F	Occasional cough	Mild bronchial pattern	Negative	n/a

## 2. Materials and methods

Cats ( $n=7$ ) inhabiting four different regions of the Balearic Island of Ibiza (Santa Eulalia, San Antonio, Es Cubells and Ses Salines) were presented for veterinary examination and were subsequently found to exhibit signs of respiratory infection (Table 1). The lungs of four cats were further radiographed to assess lung pathology. Individual faecal samples from each of six cats were pooled over three consecutive days, with the remaining cat having faeces collected from a single day. Faecal samples (5 g) were examined for the presence of first stage larvae (L1) using the Baermann method as previously described (Taubert et al., 2009). Nematode species were differentiated on the basis of size and tail morphology. Morphological measurements were made using Motic software (Images Plus v 2.0; Motic).

*Biomphalaria glabrata* snails were also experimentally infected with morpho-type 2 L1 from cat 3 to assess development to third stage larvae (L3) in a gastropod intermediate host. Six snails were each infected with 50 L1 in a six well culture plate with 5 ml water overnight. Snails were transferred to an aquarium, kept at 26 °C and fed on a diet of lettuce for 4 weeks. Snails were then killed and L3 were isolated using HCL/pepsin digestion of tissue and Baermann method. Morphological measurements were conducted as described for L1.

Individual larvae (for both L1 and L3) were washed in distilled water and DNA was extracted according to Jefferies et al. (2010). Genomic DNA from adult *O. rostratus* was also obtained from a bobcat (*Lynx rufus*) in the USA. A 1708-bp region of the 18S ribosomal RNA (rRNA) gene was amplified from individual larvae and *O. rostratus* using the primers NC18SF1 (5'-AAAGATTAAGCCATGCA-3') and NC5BR (5'-GCAGGTTACCTACAGAT-3') as previously described (Patterson-Kane et al., 2009). PCR products were purified using a QIAquick Kit (QIAGEN, Germany) and sequenced using forward and reverse primers. Sequences obtained from the larvae and *O. rostratus* were aligned with 16 other metastrongylid sequences available on the GenBank database, along with *Nematodirus battus* and *Nippostrongylus brasiliensis* as outgroup sequences, using CLUSTAL W (Thompson et al., 1994). Full details of species names and accession numbers are included in Fig. 2. Phylogenetic relationships based on the partial 18S rRNA gene (1604 positions) were determined

using neighbor-joining and maximum parsimony algorithms as part of the MEGA 4 software (Tamura et al., 2007). The close-neighbor-interchange and maximum composite likelihood algorithms were used for maximum parsimony and neighbor-joining analyses respectively. At least 1000 bootstrap replicates were used to infer statistical support at branch nodes. Percentage pairwise distances between species were further estimated using the maximum composite likelihood algorithm.

## 3. Results

All seven cats exhibited coughing, which was mild in some cases and only revealed by the owners after careful questioning. Cats 1, 2, 3 and 4 were presented for neutering and coughing was revealed on the admit consultation. In these cases the cough was mild and only cat 1 had a radiograph taken prior to anesthesia. Owners declined any trial treatment. Cat 5 had severe respiratory signs (cough, labored breathing and caquexia). Abnormalities in thorax radiographs were observed (diffuse bronchointerstitial pattern) and numerous nematode larvae in a direct fecal sample smear were noted, both suggestive of lungworm infection. Fenbendazole was administered at 20 mg/kg once a day for 21 days. A marked clinical improvement was noted and a follow up thorax radiograph (1 month after presentation) revealed a slight improvement in the lung pattern. Cats 6 and 7 were presented with cough amongst other variable signs. Thorax radiographs revealed a moderate bronchial pattern for both cats. Cat 6 recovered spontaneously without any treatment and Cat 7 was further tested negative for FeLV/FIV using an IDEXX ELISA Snap test. Further details of each case are given in Table 1.

Faecal analysis using the Baermann method identified first stage larvae in four of the seven cats (57%). Two different larval types were observed based on length and tail morphology, with one cat exhibited co-infection with both morpho-types. One morpho-type (found in 3/7 cats) was identified as *A. abstrusus* and had an approximate length of 335 (326–350  $\mu\text{m}$ ,  $n=6$ ) and characteristic tail morphology (Fig. 1A). The second morpho-type (found in 2/7 cats) was larger with an average length of 469.3  $\mu\text{m}$  (435–521  $\mu\text{m}$ ,  $n=8$ ) and differed in tail morphology when compared to *A. abstrusus*. A dorsal kink was absent and a less pronounced knob-like structure was observed at the terminal end of the tail (Fig. 1B). The second morpho-type also successfully

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