



Short Communication

New insights on metastrongyloid lungworms infecting cats of Sardinia, Italy



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ARTICLE INFO

Article history:

Received 29 November 2013

Received in revised form 29 March 2014

Accepted 1 April 2014

Keywords:

Broncho-pulmonary nematodes

Aelurostrongylus abstrusus

Troglostrongylus brevior

Cats

Epidemiology

Sardinia

ABSTRACT

In addition to the well-known *Aelurostrongylus abstrusus* (Strongylida: Angiostrongylidae), *Troglostrongylus brevior* (Strongylida: Crenosomatidae) has recently been diagnosed as a causative agent of broncho-pulmonary infections of cats in Spain and Italy. However, information concerning the impact of this species of lungworm on feline population is limited to a few case reports. From July 2011 to May 2013 an epidemiological survey was carried out on Sardinia island (Italy), where 107 individual faecal samples were examined by Baermann technique, and first-stage larvae were identified based on their morphology and characterization of molecular markers. The 29.9% (32/107) of cats examined were infested by broncho-pulmonary nematodes and, although *A. abstrusus* was the most frequently detected ($n=27$; 25.2%), larvae of *T. brevior* were also found ($n=7$; 6.5%). In addition, two cats (1.9%) were co-infested by both species. Overall metastrongyloid infection was higher in female cats ($n=22$; 38.6%) than in males ($n=10$; 20%) ($\chi^2=4.39$; $p=0.036$). The mean age of positive animals was 21.1 (± 29.8) months, being infected animals from 2 months to 10 years of age. Of the 32 animals that scored positive for lungworms only 6 (18.8%) displayed a respiratory condition associated with lungworm infestations. Biomolecular characterization confirmed the morphological diagnosis of *A. abstrusus*. Positive samples that were identified at genus level as *Troglostrongylus* spp. were molecularly characterized as *T. brevior*. This study represents the first epidemiological survey on metastrongyloid lungworms of domestic cats in Sardinia and the first report of *T. brevior* on this island.

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1. Introduction

Among the parasite species of the super-family Metastrongyloidea infecting the lungs of felids, *Aelurostrongylus abstrusus* (Strongylida: Angiostrongylidae) has for a long time been regarded as the only metastrongyloid in

domestic cats (*Felis silvestris catus*) (Anderson, 2000; Bowman et al., 2002). Indeed, pulmonary infections by *A. abstrusus* are widespread throughout most European countries with prevalence rates up to 24.4%, according to sampled populations and diagnostic procedures (Traversa et al., 2010). Nevertheless, other metastrongyloids, such as *Troglostrongylus brevior* and *Troglostrongylus subcrenatus* (Strongylida: Crenosomatidae), have been recently reported as causative agents of respiratory infection in domestic cats (Jefferies et al., 2012; Brianti et al., 2012, 2013). *Troglostrongylus* spp. lungworms have

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been described since the 1940s as parasites of wild felids (Gerichter, 1949; Sarmiento and Stough, 1956), but reported on occasion in domestic cats (Fitzsimmons, 1961; Paggi, 1959). It is yet to be defined whether their occurrence in domestic cats is sporadic, neglected or underestimated (Traversa and Di Cesare, 2013; Otranto et al., 2013). Morphological similarities of first-stage larvae (L1) of *Troglostrongylus* spp. and *A. abstrusus* shed in the faeces of infected animals make their microscopic identification challenging and may led to misidentification (Otranto et al., 2013; Brianti et al., 2014). Therefore, the combination of copromicroscopy and specific molecular diagnostic techniques has been advocated, especially for epidemiological surveys (Brianti et al., 2014).

A. abstrusus and *T. brevior* display an indirect life-cycle with terrestrial molluscs serving as intermediate hosts and amphibians, birds, reptiles, and small mammals as paratenic ones. Both species share intermediate and paratenic hosts (Gerichter, 1949), may simultaneously develop in the same snail species (i.e., *Helix aspersa*) (Giannelli et al., 2014), therefore indicating that both parasites may live in sympatry. Indeed, cases of co-infection by *A. abstrusus* and *T. brevior* have been reported in domestic cats from Spain (Jefferies et al., 2012) and Italy (Annoscia et al., 2014; Di Cesare et al., 2014). Data on the epidemiology of broncho-pulmonary nematodes of cats in Sardinia Island are scant and a prevalence of only 7.4% for *A. abstrusus* was reported in a small community of cats (Porqueddu et al., 2004) upon copro-microscopical diagnosis. This study investigates the actual role of *A. abstrusus* and *T. brevior* in determining broncho-pulmonary infections in cats from Sardinia by combining copro-microscopical and biomolecular techniques towards an understanding of the epidemiology of this little known parasitosis.

2. Materials and methods

2.1. Identification of broncho-pulmonary nematodes

From July 2012 to July 2013, a total of 107 faecal samples from domestic cats, referred for routine vaccination or clinical visit at the Veterinary Teaching Hospital of the University of Sassari, Italy were collected and examined. For each animal sampled, data on sex, age, and presence/absence of respiratory symptoms were recorded in individual files.

Faecal samples were examined for L1 of broncho-pulmonary nematodes by Baermann method using 3 g of faeces (Euzebly, 1981). Larvae were identified using morphometrical keys for *A. abstrusus* (Gerichter, 1949; Scott, 1972; Sloss et al., 1994) and for *Troglostrongylus* spp. (Gerichter, 1949; Brianti et al., 2014).

2.2. Biomolecular survey

Larvae were then stored according to their morphotype into microtubes and DNA extracted using a commercial kit (High Pure PCR Template Preparation kit, Roche Diagnostics, Mannheim, Germany). Molecular identification for *A. abstrusus* was carried out using a nested PCR assay, targeting a 233 bp region internal to the Internal Transcribed

Spacer 2 (ITS2) of ribosomal DNA (rDNA) (Traversa et al., 2008a). *Troglostrongylus* spp. isolates were processed by mitochondrial cytochrome *c-oxidase subunit 1* (*cox 1*) gene and ITS2 of ribosomal RNA gene as described by Brianti et al. (2012).

All PCR products were purified and sequenced, the sequences were then aligned using MEGA V.5.2.1 and compared with those available in GenBank using BLAST (Altschul et al., 1997).

Data on prevalence within different age classes were stratified as follows: (1) $>0 \leq 12$ months; (2) $>12 \leq 24$ months; (3) >24 months. Chi-square tests were used for comparison of the infection prevalences between the two parasite species and among age classes using Epi-Info v. 7.0 (CDC/WHO, Atlanta, GA, USA). The variance of age between infected and non-infected animals was compared with Student's *t*-test. Significance level was set for values of $p < 0.05$. All results are reported as mean \pm standard deviation or as prevalence \pm 95% confidence intervals (CI).

3. Results

Of the 107 animals examined, 32 (29.9%; CI: 21.2–38.6) scored positive for L1 for metastrongyloids the majority being infested by *A. abstrusus* ($n = 27$; 25.2%; CI: 17–33.5), followed by *T. brevior* ($n = 7$; 6.5%; CI: 1.9–11.2). The difference between the prevalence rates of the two nematodes was statistically significant ($\chi^2 = 13.99$; $p < 0.001$). Among infected animals, two kittens (6.3%) were simultaneously co-infected by both metastrongyloid species. *A. abstrusus* and *T. brevior* L1 larvae were morphological differentiated based on their caudal end, oral opening, and body length (Fig. 1).

Molecular identification of *A. abstrusus* (JX948745.1) showed a 100% homology among them ($n = 27$) and a single nucleotide difference (at 393 bp residue; 99% homology) with sequences of *A. abstrusus* isolates (DQ372965.1; EU034168.1) from continental Italy. Genomic sequences of *T. brevior* showed a 100% homology with *cox1* and ITS2 sequences of *T. brevior* deposited in GenBank (KF270684 and KF241978, respectively).

The mean age of positive cats (21.1 ± 29.8 months) was not statistically significantly different ($p = 0.963$) to that of negative ones (mean 20.8 ± 35.1 months). The same applies to each age group (χ^2 for linear trend = 0.000; $p = 0.990$), as shown in Table 1. The age of infected cats was 23.6 ± 31.6 months for *A. abstrusus*, and 8.1 ± 8.0 months for *T. brevior*. Metastrongyloid infections were statistically more frequent in females (38.6%) than in males (20%) ($\chi^2 = 4.39$, $p = 0.0361$). The majority of infested animals did not display clinical signs, whilst six infested cats (18.8%) showed respiratory symptoms (i.e., coughing and nasal discharge). No significant differences have been observed between the rate of respiratory symptoms in *T. brevior* (28.6%) and in *A. abstrusus* (18.5%) infested cats (Yates corrected = 0.0038; $p = 0.95$).

4. Discussion

This study represents the first comprehensive survey on metastrongyloid lungworms infecting cats in Sardinia,

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