OCCURRENCE OF LUNGWORMS IN EUROPEAN WILDCATS (FELIS SILVESTRIS SILVESTRIS) OF CENTRAL ITALY

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ABSTRACT: The increasing focus on infections in domestic cats (Felis catus) has raised questions about lungworm distribution in wild hosts. To enhance knowledge of the occurrence of lungworms in enzootic regions of central Italy, we examined the carcasses of 16 European wildcats (Felis silvestris silvestris). Adult nematodes, feces, respiratory flushings, and pulmonary tissues were collected at necropsy and then microscopically and genetically analyzed. Fourteen wildcats had single or mixed lungworm species. Aelurostrongylus abstrusus was the most common parasite retrieved, followed by Troglostrongylus brevior. In addition, three specimens of Angiostrongylus chabaudi were found in the pulmonary arteries of one wildcat. Histologically, the most common lesions were a mild-to-severe chronic catarrhal bronchitis and a chronic interstitial pneumonia with smooth muscle hypertrophy, associated with T. brevior and A. abstrusus, respectively. These results demonstrate that the European wildcats may harbor several species of lungworms that may impair their health and welfare. Also, F. s. silvestris is a potential reservoir for respiratory nematodes in domestic cats.

Key words: Aelurostrongylus abstrusus, Capillaria aerophila, Felis silvestris silvestris, Italy, Troglostrongylus brevior.

INTRODUCTION

Various parasitic nematodes, called lungworms, infect the respiratory system of domestic and wild felids. The metastrongyloid Aelurostrongylus abstrusus (cat lungworm) is the most important in terms of its geographic distribution and role in respiratory diseases of domestic cats (Felis catus) (Traversa and Di Cesare 2013). This nematode also has been reported in wild felids, but most records have not been supported by valid microscopic or genetic evaluations (Traversa 2014).

Metastrongyloids within the genus Troglostrongylus are regarded as occasional lungworms infecting wild felids (e.g., the African wildcat, Felis silvestris libyca [formerly Felis ocreata]; bobcat, Lynx rufus; and Panthera spp.; Brianti et al. 2014). However, descriptions of Troglostrongylus brevior from domestic cats stimulated a new scientific interest in this parasite and raised issues about its host affiliation (Brianti et al. 2012; Orranto et al. 2013; Traversa and Di Cesare 2013).

The trichuroid Capillaria aerophila (syn. Eucoleus aerophilus) can infect domestic and wild animals, including some felids (e.g., bobcats and wildcats; Krone et al. 2008; Traversa and Di Cesare 2013). The number of cases of lung capillariosis in domestic cats has increased, albeit the true geographic distribution of C. aerophila in domestic hosts is still poorly known (Traversa et al. 2010).

Knowledge of host affiliation and distribution of lungworms needs to be improved, toward a better understanding of the potential epidemiologic role of wild felids as a source of bridging infections with domestic cats. In the past decade feral and wild felids were incriminated, along with other factors, as a major source of infections in pets with emergent cardiopulmonary nematodes (Traversa et al. 2010). Moreover, molecular results demonstrated that genetically identical populations of these parasites can be shared.
between wildlife and pets (Di Cesare et al. 2014b; Eleni et al. 2014).

Biological (e.g., expansion of intermediate hosts) and phenologic (e.g., global warming, environmental changes) modifications for lungworms and their wild hosts could nurture an apparent emergence of these nematodes in domestic cats (Traversa and Di Cesare 2014).

Current populations of the European wildcat (*Felis silvestris sylvestris*) are irregularly distributed in various countries. In Italy, this felid is present in the Eastern Sub-alps and Alps, the Central-southern Sub-Apennine and Apennine range, and Sicily (Ragni et al. 2012). European wildcats and the domestic cats can live in sympatry, interbreed, and produce fertile offspring, yet hybridization is negligible and mainly occurs at the ecologic edges of wildcat populations (Lecis et al. 2006; Mattucci et al. 2013). This implies that where wild and domestic cats share the same ecologic niche, they may be at the same risk of infection for parasites.

Two studies demonstrated *T. brevior* or *C. aerophila* in domestic cats and a few hybrids from limited regions of northern and southern Italy (Beraldo et al. 2014; Falsone et al. 2014). Nevertheless, data on the presence of lungworms in wildcats living in central Italy are lacking. This knowledge gap is important from an epizootiologic viewpoint, given that wildcat populations in the central region occur at relatively high densities (Ragni 2006; Anile et al. 2014). In addition, coastal areas and Apennine territories of central Italy have the highest number of single clinical cases and prevalence of cat lungworms in Europe (Traversa et al. 2008, 2009; Di Cesare et al. 2011, 2014a).

We documented the occurrence of lungworms in wildcats from selected regions of central Italy to provide new insight into the significance of these little-known and apparently emerging parasites.

**MATERIALS AND METHODS**

**Animals**

Sixteen carcasses of road-killed animals consistent with European wildcats were collected from various provinces of regions of central Italy (Table 1). The collection was opportunistic and cumulative; it occurred between December 2008 and April 2014 as sporadic collection of animals donated by collaborating research groups.

Standard data concerning sex, estimated age (based on genitalia, proportion and size of body and dentition), general condition, head and body, hind foot and tail lengths, and weight (Ragni and Possenti 1996) were collected. The identification of each individual as *F. s. silvestris* was based on external coat pattern, and other morphologic characteristics, including intestine and cranial indexes (Ragni and Possenti 1996). The identity of some wildcats that showed physical patterns compatible with hybrids with domestic cats was confirmed by genetic analysis using autosomal microsatellite markers (Randi et al. 2001).

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<tr>
<th>Sex</th>
<th>Age</th>
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<tr>
<td>Juvenile</td>
<td>Male 8 mo</td>
<td>Marche</td>
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<td>&lt;1 yr</td>
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<td>Adult</td>
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<td>Female</td>
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<td>Male</td>
<td>4 yr</td>
<td>Lazio</td>
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**Necropsy, parasite collection, and morphologic identification**

All collected animals were necropsied. In some cases carcasses were incomplete due to traumatic injuries from car accidents or possible predation. However, the entire cardiorespiratory tract was present in all animals. Trachea, bronchi, heart, and pulmonary arteries were opened and checked for visible adult nematodes. When gross lesions were observed, parts of the lung lobes were checked for parasites using a stereomicroscope and dissected with forceps. Lungs were soaked in 0.85% saline
solution for 8 h. The liquid was then filtered through a 500-μm sieve and centrifuged at 1,500 × G for 10 min. The sediments were examined using a stereomicroscope, and aliquots were preserved at −20 °C pending molecular analysis.

All adult nematodes from the airways were washed and either stored in 70% ethanol (ETOH) pending further analysis or mounted on slides to be immediately observed microscopically. The parasites were identified morphologically according to key features (Gerichter 1949; Fitzsimmons 1961; Skrjabin et al. 1992) and individually stored in 70% ETOH for further genetic analysis. A fecal sample was collected from the last tract of the colon of each wildcat and divided into two aliquots. The first aliquot was subjected to a copromicroscopic examination (Sloss et al. 1994) to look for capillarid eggs and nematode larval stages. All parasite elements were identified morphologically according to key features (Gerichter 1949; Brianti et al. 2012; Di Cesare et al. 2012a; Traversa and Di Cesare 2013). The second aliquot was stored at −20 °C pending molecular investigations. A wildcat was considered to be infected with lungworms if any test revealed parasites.

**Histopathological examinations**

Due to poor conservation status of four animals, tissues for histologic examination were sampled from the lungs of 12 of the 16 carcasses. Samples were collected from gross lesions, if present, or in standard anatomic sites (i.e., basal, middle, and cranial lobes of each lung). Tissues were fixed in neutral 10% buffered formalin, routinely processed, paraffin embedded, cut into 5-μm sections, and stained with H&E.

**Molecular procedures**

Adult parasites (23 nematodes) and larvae recovered from feces (15 samples), pulmonary tissues (16 samples), and respiratory lavages (11 nasal and 16 lung lavages) were subjected to molecular examination. Genomic DNA was individually extracted from adult parasites and pulmonary tissues by using the QIAamp Tissue Kit (QIAGEN GmbH, Hilden, Germany) after disruption with liquid nitrogen. Genomic DNA was individually extracted from larval and flushing samples by using the QIAampDNA stool Mini Kit (QIAGEN) after three freeze-thaw cycles with liquid nitrogen for 5 min and at 95 °C for 5 min.

The internal transcribed spacer 2 (ITS2) regions of single adult lungworms were amplified using universal primers for nematodes, namely, NC1 (5′-ACGTCCTGTTACGGGTTTGT-3′; forward) and NC2 (5′-TTAGTTCTTCTTGCCTCGCT-3′; reverse) (Chilton 2004), 5 μL of template, 25 μL of REDTaq® ReadyMix™ (Sigma-Aldrich, St. Louis, Missouri, USA), and rinsed with distilled water provided by the same manufacturer. Reactions were performed on a 2700 thermocycler (Applied Biosystems, Waltham, Massachusetts, USA) as follows: 95 °C for 10 min and 40 cycles at 94 °C for 60 s, 50 °C for 60 s, 72 °C for 60 s, followed by a final extension for 10 min at 72 °C.

DNA samples extracted from feces and flushings were examined with two nested PCRs amplifying specific regions internal to the ITS2 of A. abstrusus (~233 base pairs [bp]) and T. brevior (~356 bp) as described by Di Cesare et al. (2014a). The optimal conditions of the PCR mixtures of the first round were the same as for the amplification of the ITS2 from adult stages, whereas those for the second round were as follows: 50-μL reaction containing 200 pmol of each TbrFor and TbrRev primer, 4 μL of a 1:20 dilution of each NC1-NC2 amplicon, 25 μL of REDTaq Ready Mix, and rinsed with distilled water. The cycling protocol used in both rounds was the same as that used for adult parasites, with slight modifications.

The DNA extracts from pulmonary lavages were also subjected to a seminested PCR protocol specific for a 299-bp diagnostic region within the cox1 gene of C. aerophila, with slight modifications of a previous protocol (Di Cesare et al. 2012b). In the first step, primers Cox1NEMS and Cox1NEMR were used, whereas the forward primer CaerInt2F (5′-GAAGCCCTTAATAACTAT TTCAGG-3′) was used in the second round. PCR mixtures (50 μL) contained 100 pmol of each primer in both steps, 4 μL of DNA extract in the first step and 5 μL of template (1:20) in the second step, 25 μL of REDTaq ReadyMix, and distilled water. Reactions were performed as follows: 95 °C for 10 min and 40 cycles at 94 °C for 60 s, 48 °C (first step) or 52 °C (second step) for 60 s, 72 °C for 60 s, followed by a final extension for 10 min at 72 °C. The amplicons were purified and sequenced. The sequences were aligned using Data Analysis in Molecular Biology and Evolution version 4.5.55 (Xia and Xie 2001) and compared with those of the DNA of other nematodes available in GenBank by using the nucleotide-nucleotide Basic Local Alignment Search Tool (NCBI 2015).

**RESULTS**

**Infection rates and parasite identifications**

Six juveniles (two females, four males) and 10 adults (one female, nine males), with body weights from 1.6 to 3.9 kg, were examined (Table 1). Fourteen (88%) were positive for at
least one lungworm. The most prevalent species was *A. abstrusus* (63%) followed by *T. brevior* (50%) and *C. aerophila* (19%). Nine animals had a monospecific infection either by *A. abstrusus* (31%), *T. brevior* (19%), or *C. aerophila* (13%). Three (19%) cats had a mixed infection by *A. abstrusus* and *T. Brevior*; one (6%) was infected by *T. brevior* and *C. aerophila*; and one (6%) was positive for *A. abstrusus*, *T. brevior*, and *C. aerophila*. Moreover, three nematodes were found in the pulmonary arteries of this latter wildcat, but their poor condition allowed only a microscopic identification as *Angiostrongylus* sp.; nonetheless, they were identified as *Angiostrongylus chabaudi* based on genetic data (Traversa et al. 2015).

Molecular characterization corroborated the microscopic identification, as sequences of *A. abstrusus*, *T. brevior*, and *C. aerophila* showed a 99–100% homology with those available in GenBank (JX290564.1, EU034168.2, and JQ905052.1, respectively).

Histologic examinations

All cats positive for *T. brevior* that were examined histopathologically (six of eight) had a moderate-to-severe catarrhal bronchitis affecting medium and large bronchi. Four of these animals harbored a mixed infection of *T. brevior* and *A. abstrusus*, plus *C. aerophila* in one wildcat. In addition, catarrhal bronchitis was observed in three cats that were infected only with *A. abstrusus*. The inflammation involved both large- and medium-sized bronchi whose lumina were partially to almost entirely filled with mucus and smudged epithelial cells. In some cases only a mild peribronchial lymphocytic infiltrate was present. Chronic bronchitis seemed more severe when *A. abstrusus* was present (Fig. 1).

Interstitial pneumonia and vascular damage occurred primarily in wildcats infected with *A. abstrusus*. Interstitial pneumonia that occurred in five of 12 cats also was associated with catarrhal pneumonia in four cats. Severity of the pneumonia ranged from a focal accumulation of mononucleated cells (lymphocytes and macrophages) in alveolar septa to diffuse thickening of septa by mixed inflammatory infiltrate composed of lymphocytes and, to a lesser extent, eosinophils and macrophages. Four of these cats were infected with *A. abstrusus*: three with single infection and one with mixed infection by *T. brevior*. The primary infiltrate of macrophages and lymphocytes was focused around adult nematodes, larvae, and eggs. These lesions were dispersed throughout lung tissue, but mostly in subpleural sites. These lesions were found in one wildcat infected only by *T. brevior*.

A diffuse hypertrophy of smooth muscle of arterioles (medial hypertrophy) (Figs. 2, 3A) and of muscular septa in the lung parenchyma...
was observed in five animals. In three cats, muscle hypertrophy was associated with intralesional *A. abstrusus* (Fig. 3A, B). No parasites were evident in the histologic sections of the other two cats, but these animals were positive for *A. abstrusus*. The smooth muscle hypertrophy was associated with interstitial pneumonia in four cats and was the only histologic finding in one cat. Infiltration by neutrophils and lymphocytes, with degeneration of muscular and endothelial cells (vasculitis), was found in one of these animals.

**DISCUSSION**

Our results show that the cat lungworm *A. abstrusus* can occur in *F. s. silvestris* with high infection rates and significant pulmonary lesions. Previous reports described *Aelurostrongylus* spp. or *A. abstrusus* in cheetahs (*Acinonyx jubatus*; West et al. 1977), lions (*Panthera leo*; Bjork et al. 2000), and the Amur leopard cat (*Prionailurus bengalensis*; Gonzáles et al. 2007). Therefore, this parasite is considered capable of infecting wild felids (Otranto et al. 2013). Nevertheless, its factual occurrence in hosts other than the domestic cat was unclear (Traversa 2014). For example, measures of first-stage larvae regarded as *Aelurostrongylus* spp. from the feces of lions (Bjork et al. 2000) or identified as *A. abstrusus* from an Amur cat (Gonzáles et al. 2007) were consistent with *Troglostrongylus* spp. (Traversa 2014). Almost no information is available on *A. abstrusus* in wildcats. Despite histologic findings suggesting aelurostrongylosis, parasites found in an *F. s. silvestris* in Portugal were not described (Travassos et al. 2010). *Aelurostrongylus* spp. was detected in few wildcats necropsied in northern Italy (Beraldo et al. 2014), but not in wildcats from southern Italy (Falsone et al. 2014) or Germany (Krone et al. 2008). Regardless, our study demonstrates that, in some enzootic regions, *A. abstrusus* occurs with high prevalence in European wildcats.

*Troglostrongylus brevior* has been identified in wildcats in other geographic regions of Italy (Beraldo et al. 2014; Falsone et al. 2014) and central Europe (Steeb et al. 2014). Also, *T. brevior* has been increasingly detected in domestic cats, and issues have been raised on the origin and current distribution of *Troglostrongylus* spp. in domestic hosts (Otranto et al. 2013; Traversa and Di Cesare 2013; Falsone et al. 2014; Traversa 2014). After the first descriptions of *T. brevior* in *F. s. libyca* (Gerichter 1949), it was recorded only in a wildcat and a feral domestic cat from Italy.
and in domestic cats from the European Islands of Ibiza, Sardinia, Sicily, and Crete (Jeffries et al. 2010; Brianti et al. 2012; Diakou et al. 2014; Tamponi et al. 2014) and in central and southern Italy (Brianti et al. 2013; Di Cesare et al. 2014a; Traversa et al. 2014). Some studies (Beraldo et al. 2014; Falsone et al. 2014; Steeb et al. 2014; this study) suggest that the European wildcat is the natural host of *T. brevior* and that spillover to domestic cats may occur in some enzootic areas.

Although *C. aerophila* has been found several times in domestic cats (Traversa et al. 2009; Di Cesare et al. 2011, 2012b) even in coinfections with *T. brevior* or *A. abstrusus* (Di Cesare et al. 2015b), little is known about its occurrence in wild felids. It was found in European wildcats from Germany (Krone et al. 2008) and southern Italy (Falsone et al. 2014) and northern Italy (Beraldo et al. 2014). The absence of *C. aerophila* in most cats from areas where prevalence of capillarids in domestic carnivores is high (Traversa et al. 2009, 2012; Di Cesare et al. 2011; Veronesi et al. 2013, 2014a, b) suggests that the European wildcat is not a primary host for *C. aerophila*. However, *F. s. silvestris* can harbor this parasite, although other wildlife such as foxes and mustelids may play a primary role in the epizootiology of lung capillariosis (Di Cesare et al. 2014b).

*Angiostrongylus chabaudi* was found only in wildcats from central Italy, whereas domestic cats, dogs, foxes, and badgers living in the same areas were negative (Biocca 1957). Then, it remained practically unknown until the past 2 yr, when it was isolated in two domestic cats: one from Sardinia, Italy (Varcasia et al. 2014), and one from the Umbria region of central Italy (Traversa et al. 2015), the same region from which the wildcat infected in this study was obtained. In addition, this latter cat was also infected by *T. brevior* and *A. abstrusus* (Traversa et al. 2015). Further studies are warranted to evaluate the occurrence of the apparently rare *A. chabaudi* in wild and domestic felids from Italy and elsewhere.

The reasons for the findings of the rare felid parasites *T. brevior* and *A. chabaudi* in unusual hosts are unknown. The current scenario suggests a spillover from wildcats to domestic cats and vice versa, causing the presence of *T. brevior* and *A. chabaudi* in domestic cats and of *A. abstrusus* in wildcats. A retrospective study documented that the occurrence of *T. brevior* in domestic cats was formerly negligible (Di Cesare et al. 2015a).

Changes in climate and in the ecology of intermediate and definitive hosts could be the basis of modifications in the epizootiology of metastrongyloid lungworms. As a key example, *Angiostrongylus vasorum* is emerging in dogs from various regions of Europe, perhaps as a result of change in the habitat of red foxes (*Vulpes vulpes*), thereby leading to expansion of this parasite in wolves and dogs (Morgan et al. 2009; Morgan and Shaw 2010; Eleni et al. 2014). A similar situation may apply to local populations of wildcats and lungworms. Modifications in demographic and ecologic conditions are predicted to increase the potential for cross-breeding between wild and domestic cats (Randi et al. 2001; Pierpaoli et al. 2003; Lecis et al. 2006). Reduction of woodland and urbanization of sylvatic territories in mountainous and hilly territories of Italy favored a progressive enlargement of the peninsular range of the European wildcat (Ragni et al. 2010). Simultaneously, a massive and uncontrolled diffusion of stray and free-ranging domestic cats has been observed in urban and periurban settlements (Petruzzi et al. 2014). These changes have the potential to promote contact between wild and domestic cats in the same habitat and thus potential sharing of disease-causing pathogens (Krone et al. 2008), with a consequent change in the helminthofauna of felid populations, including *T. brevior*. Thus, although wildcats are its primary host, *T. brevior* is able to change hosts under suitable conditions affecting development and transmission. This may occur mostly in confined areas (e.g., islands or mountainous regions) where local factors such as occupation of the same ecologic niche and presence of susceptible intermediate
hosts may nurture the risk of bridging infection between wildcats and domestic cats.

Haplotype 1 of C. aerophila, as seen in our study, is the most common in terms of geographic distribution in Europe and is shared by wildlife and companion animals, including domestic cats (Di Cesare et al. 2014b). This further corroborates the existence of common patterns of transmission of lungworms between wildcats and domestic hosts.

Gross and histologic findings of A. abstrusus infection in cats are well known, whereas this information for Troglostrongylus spp. is scanty. Single case reports have described chronic catarrhal bronchitis of large- and medium-sized bronchi together with scattered hemorrhages in pulmonary parenchyma (Brianti et al. 2012; Traversa et al. 2014). This study documents the association of T. brevior with catarrhal bronchitis in some hosts. The lesions we found in wildcats with aelurostrongylosis are consistent with chronic infection in domestic hosts (Stockdale 1970; Caswell and Williams 2007). With regard to vascular lesions, the association of smooth muscle hypertrophy with A. abstrusus infection is debated (Maxie and Robinson 2007). Some wildcats infected with A. abstrusus displayed medial hypertrophy, and this lesion was never observed in negative cats. In some cats, hypertrophic arteries were found, with differing degrees of adventitial and intramural inflammatory infiltrates consistent with vasculitis. These findings further support chronic infection of wildcats with A. abstrusus.

In conclusion, we demonstrate that A. abstrusus can infect the European wildcat, sometimes in association with severe lung damage. Also, T. brevior can occur with high prevalence in F. s. silvestris, that is, in all likelihood, its primary host. However, in the near future, infection of domestic cats with T. brevior could occur in the absence of wildcats.

LITERATURE CITED


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