The Veterinary Journal 203 (2015) 233–238

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Retrospective study on the occurrence of the feline lungworms *Aelurostrongylus abstrusus* and *Troglostrongylus* spp. in endemic areas of Italy

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ARTICLE INFO

Article history:
Accepted 10 December 2014

Keywords:
Aelurostrongylus abstrusus
Troglostrongylus spp
Cat
PCR
Epidemiology

ABSTRACT

Aelurostrongylus abstrusus is a metastrongyloid nematode infesting the respiratory system of domestic cats worldwide. *Troglostrongylus brevior* and *Troglostrongylus subcrenatus*, two lungworms thought to infest wild felids, have been found recently in domestic cats from Spain and Italy. These unexpected findings have raised doubts about the assumed past and present occurrence of *Troglostrongylus* spp., especially *T. brevior*, in domestic hosts and suggest that there may have been missed detection or misdiagnosis. The present retrospective study evaluated the presence of lungworms in cats from Italy with a diagnosis of respiratory parasitism or with compatible lung lesions from 2002 to 2013. Sixty-eight samples of DNA and larvae from cats with a diagnosis of aelurostrongylosis, and 53 formalin-fixed paraffin-embedded lung samples from cats confirmed as lungworm infested or with compatible lesions, were investigated using two DNA-based assays specific for *A. abstrusus* or *T. brevior*. All DNA and larval samples were positive for *A. abstrusus* and one was additionally positive for *T. brevior*. Most paraffin-embedded lung tissues were positive only for *A. abstrusus*, but two samples tested positive for both lungworms and one for *T. brevior* only. This study supports the major role of *A. abstrusus* in causing feline respiratory parasitism in endemic areas of Italy.

Introduction

The feline lungworm *Aelurostrongylus abstrusus* (Metastrongyloidea, Angiostrongylidae) affects the respiratory system of domestic cats (*Felis silvestris catus*) worldwide. Infestation may be subclinical or result in respiratory signs, such as coughing, dyspnoea and tachypnoea (Traversa et al., 2008a, 2010; Traversa and Di Cesare, 2013). Recent data suggest an increased geographical distribution of this parasite, possibly due to epidemiological and biological factors, such as modification of the phenology of mollusc intermediate hosts and altered developmental rate related to climate changes (Traversa et al., 2010; Barutzki and Schaper, 2013; Traversa and Di Cesare, 2013).

New records of unusual lungworms in domestic cats have raised the question as to whether past reports were erroneously attributed to *A. abstrusus* (Brianti et al., 2012; Otranto et al., 2013). Nematodes belonging to the genus *Troglostrongylus* (Metastrongyloidea, Crenosomatidae) previously have been considered to be associated only with wild felids; however, recently, *Troglostrongylus brevior* and *Troglostrongylus subcrenatus* have been reported to cause bronchopneumonia in domestic cats (Brianti et al., 2012, 2014; Traversa and Di Cesare, 2013).

T. brevior was first described from the respiratory system of wild felids from Palestine (Gerichter, 1949). It was then reported in a European wild cat (*Felis silvestris silvestris*) and in a feral cat caught in central Italy, but of unknown origin (Paggi, 1959). Recently, this nematode has been described in Ibiza, Spain (Jefferies et al., 2010), in Italy (Sicily and Sardinia and the Apennine region) (Brianti et al., 2012, 2013; Di Cesare et al., 2014a, 2014b; Tamponi et al., 2014) and in Crete (Diakou et al., 2014). Until it was recently described

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http://dx.doi.org/10.1016/j.tvjl.2014.12.010
1090-0233/

in a cat from Sicily (Brianti et al., 2012), *T. subcrenatus* had only been reported from a single cat in Malawi in 1961 (Fitzsimmons, 1961).

The close morphological similarities in the first larval stage (L1) shed by infested felids and discrepancies in the length of the larval body published in some papers have promoted the hypothesis that *T. brevior* may have been confused with *A. abstrusus* in the past (Jefferies et al., 2010; Otranto et al., 2013; Traversa and Di Cesare, 2013; Traversa, 2014). Common features in the biological cycle of *A. abstrusus* and *T. brevior*, such as mollusc intermediate hosts and small animal paratenic hosts (Gerichter, 1949; Di Cesare et al., 2013; Giannelli et al., 2013), and recent cases of co-infestations (Jefferies et al., 2010; Annoscia et al., 2014; Di Cesare et al., 2014a, 2014b), would support this possibility.

Although misdiagnosis of *T. brevior* infestations as *A. abstrusus* infestations is not supported by the literature (Traversa and Di Cesare, 2013; Traversa, 2014), it is a possibility, since *Troglostrongylus* spp. infestation in domestic cats was ignored prior to 2010 and there is almost no information on this infestation in cats. This is of importance considering that some studies have evaluated the efficacy of parasiticides based on detection and identification of larvae shed by cats before and after treatment (Traversa et al., 2009a, 2009b; Iannino et al., 2013). In addition, laboratory and field studies carried out with a DNA-based assay specific for *A. abstrusus* (Traversa et al., 2008b and c) may have missed the occurrence of *T. brevior*.

New molecular data have been generated for *T. brevior*, with characterisation of the 18S rRNA gene and the internal transcribed spacer 2 (ITS2) region of ribosomal DNA (Jefferies et al., 2010; Brianti et al., 2012). A duplex PCR was able to discriminate *A. abstrusus* and *T. brevior* using larvae isolated from the faeces of a cat with a mixed infestation (Annoscia et al., 2014). A nested PCR specific for *T. brevior* has been used to identify the nematode using faecal L1s and pharyngeal swabs in mixed infestations with *A. abstrusus* (Di Cesare et al., 2014a, 2014b).

With the aim to clarify whether cases of incorrect or missed diagnosis have occurred in previous cases of feline lungworm infestations in endemic areas of Italy, this study evaluated the presence of *A. abstrusus* and/or *T. brevior* in (1) DNA and larval samples from previous studies and (2) paraffin-embedded lung samples from cats submitted for postmortem examination.

Materials and methods

Sources of samples

Forty-five DNA samples were used from two studies carried out in 2007–2008 to validate a nested PCR specific for the ITS2 of *A. abstrusus* (study 1: 22 faecal samples, 7 pharyngeal swabs; Traversa et al., 2008b) and to investigate its usefulness in the field (study 2: 16 pharyngeal swabs; Traversa et al., 2008c). All samples had been stored at –20 °C at the Laboratory of Molecular Parasitology, Faculty of Veterinary Medicine, Teramo, Italy.

Larvae (L1) obtained by Baermann sedimentation from cats diagnosed with aelurostrongylosis and included in two parasiticide efficacy studies (studies 3 and 4; Traversa et al., 2009a, 2009b, respectively) had been stored since 2008 at the Laboratory of Molecular Parasitology, Faculty of Veterinary Medicine, Teramo, Italy. Genomic DNA was extracted from 23 samples as previously described (Traversa et al., 2008b).

Fifty-three formalin-fixed, paraffin-embedded blocks of lung were collected at postmortem examination from cats with a definitive diagnosis of lungworm or compatible respiratory lesions from the Pathological Anatomy Unit (UPA) of the Faculty of Veterinary Medicine, Teramo, Italy (2002–2005: $n = 4$ samples), the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM), Teramo, Italy (2008–2013: $n = 30$ samples) and the Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana (IZSLT), Rome, Italy (2008–2013: $n = 19$ samples). Genomic DNA was extracted from five to seven 5–20 µm thick sections from each block using a commercial kit (QIAamp Tissue Kit, Qiagen). Five sections from four different blocks, obtained at postmortem examination of a cat with confirmed mixed infestation with *A. abstrusus* and *T. brevior* (Traversa et al., 2014), were included as a positive control.

Molecular analysis

Samples from studies 1 and 2 were tested with a nested PCR specific for a ~233 base pair (bp) region internal to ITS2 of *A. abstrusus* (Traversa et al., 2008b). The same PCR assay was performed using DNA extracted from L1s from studies 3 and 4 and from histopathological sections. All samples were tested with a nested PCR specific for a ~356 bp region internal to the ITS2 of *T. brevior* (Di Cesare et al., 2014a). All extracts, depending upon the amount of DNA available, were tested in each PCR protocol in duplicate or triplicate. Twenty selected amplicons from each of studies 1–4 and from histopathological sections were selected for sequencing to confirm the specificity of the PCRs (Traversa et al., 2008b; Di Cesare et al., 2014a). The sequences obtained were aligned using Data Analysis in Molecular Biology and Evolution version 4.5.55 (DAMBE)[1] and compared with those of the DNA of other metastrongylid nematodes available in GenBank using BLAST.[2] Sequences of PCR amplicons for *A. abstrusus* were compared with GenBank sequences EU034168.2 DQ372965.2 and JX948745.1, while sequences of PCR amplicons for *T. brevior* were compared with GenBank sequences JX290564.1 and KF241978.1.

Results

Molecular analysis

In DNA samples extracted from faeces and pharyngeal swabs in studies 1 and 2, 42/45 (93%) samples were positive by PCR for *A. abstrusus*. One of these samples (from a cat in study 2) was also positive for *T. brevior*. The remaining three samples were PCR negative for both parasites. All 23 DNA samples extracted from larvae (L1) collected in studies 3 and 4 were positive by PCR for *A. abstrusus*, while none was positive for *T. brevior*.

In DNA extracted from formalin-fixed paraffin-embedded tissues collected from cats at postmortem examination and stored at UPA, IZSAM and IZSLT, 51/53 (96%) were positive by PCR for *A. abstrusus* and 3/53 (6%) were positive by PCR for *T. brevior* (Table 1). Two samples were positive by PCR for both *A. abstrusus* and *T. brevior*, while one sample was positive for *T. brevior* only. One sample was negative by PCR for both parasites. Sequence analysis confirmed the identity of the products amplified by *A. abstrusus* and *T. brevior*-specific PCRs.

Postmortem findings

A variety of different lungworm stages were observed histologically in sections of lung from all 19 cats at IZSLT and 23/30 cats at IZSAM; no nematodes were observed in histological sections of lung from the four cats at UPA (Table 2). However, histological lesions consistent with lungworm infestation were found in the remaining seven cats at IZSAM (three of these cats also had lungworm infestation confirmed by Baermann examination) and all four cats at UPA.

Lung lesions compatible with verminous pneumonia, including granulomatous foci containing parasitic stages, were observed histologically (Table 2). Most adult lungworms were present in the alveoli and bronchioles (Tables 1 and 2). Of the two cats positive for *A. abstrusus* and *T. brevior*, one harboured adult stages in both bronchioles and bronchi (cat 39; Fig. 1a), while the other (cat 47) had larvae and eggs only in the alveoli. The cat positive only for *T. brevior* (cat 49; Fig. 1b) had adult stages only in a bronchiole. Cat 35, which was PCR positive only for *A. abstrusus*, harboured adult stages in the bronchi. Samples from cat 38, which were PCR negative for both *A. abstrusus* and *T. brevior*, had evidence of lung damage, nematode eggs and larvae ~230–255 µm long and ~15 µm wide on histological examination (Fig. 1c). Verminous pneumonia was considered to be the cause of death of 11/53 (21%) cats at postmortem examination (10 at IZSAM and one at IZSLT); most of these cats were <1 year of age (Tables 1 and 2). In 5/53 cats, the cause of death was concomitant parasitic and bacterial pneumonia.

[1] See: http://dambe.bio.uottawa.ca/software.asp (accessed 15 May 2014).
[2] See: http://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed 15 May 2014).

Table 1
PCR results for *Aelurostrongylus abstrusus* or *Troglostrongylus brevior* in paraffin-embedded sections of feline lungs obtained at postmortem examination in Italy (2002–2013).

Cat	Year	Geographical origin	Age	*Aelurostrongylus abstrusus*	*Troglostrongylus brevior*
UPA					
1	2002	Ascoli Piceno, Marche region	4 years	+	–
2	2002	Pescara, Abruzzi region	1.5 years	+	–
3	2002	Teramo, Abruzzi region	2 months	+	–
4	2003	Ascoli Piceno, Marche region	4.5 years	+	–
IZSAM					
5	2008	Teramo, Abruzzi region	Adult	+	–
6	2008	Teramo, Abruzzi region	Adult	+	–
7	2008	Teramo, Abruzzi region	50 d	+	–
8	2009	Teramo, Abruzzi region	NA	+	–
9	2009	Chieti, Abruzzi region	Adult	+	–
10	2009	Teramo, Abruzzi region	NA	+	–
11	2009	Chieti, Abruzzi region	NA	+	–
12	2009	Chieti, Abruzzi region	12 years	+	–
13	2009	Chieti, Abruzzi region	3 months	+	–
14	2009	Pescara, Abruzzi region	NA	+	–
15	2009	Teramo, Abruzzi region	NA	+	–
16	2009	Teramo, Abruzzi region	NA	+	–
17	2011	Teramo, Abruzzi region	16 years	+	–
18	2011	Teramo, Abruzzi region	11 years	+	–
19	2011	Teramo, Abruzzi region	7 years	+	–
20	2012	Avezzano, Abruzzi region	1–2 years	+	–
21	2012	Teramo, Abruzzi region	NA	+	–
22	2012	Chieti, Abruzzi region	Adult	+	–
23	2012	Teramo, Abruzzi region	1 year	+	–
24	2012	Campobasso, Molise region	1 year	+	–
25	2012	Teramo, Abruzzi region	6 months	+	–
26	2012	Teramo, Abruzzi region	5 years	+	–
27	2012	Pescara, Abruzzi region	4 months	+	–
28	2012	Pescara, Abruzzi region	4 years	+	–
29	2012	Teramo, Abruzzi region	20 years	+	–
30	2012	Teramo, Abruzzi region	NA	+	–
31	2012	Teramo, Abruzzi region	4 months	+	–
32	2012	Teramo, Abruzzi region	2 months	+	–
33	2012	Teramo, Abruzzi region	2 years	+	–
34	2012	Teramo, Abruzzi region	5 years	+	–
IZSLT					
35	2008	Grosseto, Tuscany region	NA	+	–
36	2008	Rome, Latium region	9 months	+	–
37	2008	Rome, Latium region	5 months	+	–
38	2008	Rome, Latium region	2 months	–	–
39	2010	Rome, Latium region	5 years	+	+
40	2010	Rome, Latium region	7 months	+	–
41	2010	Rome, Latium region	7 months	+	–
42	2011	Rieti, Latium region	2 years	+	–
43	2011	Rome, Latium region	1 year	+	–
44	2011	Rome, Latium region	4 years	+	–
45	2011	Grosseto, Tuscany region	1 year	+	–
46	2011	Rome, Latium region	Aged	+	–
47	2012	Rome, Latium region	5 years	+	+
48	2012	Rome, Latium region	5 years	+	–
49	2013	Rome, Latium region	1 year	–	+
50	2013	Rieti, Latium region	Adult	+	–
51	2013	Viterbo, Latium region	Adult	+	–
52	2013	Rome, Latium region	3 months	+	–
53	2013	Latina, Latium region	6 months	+	–

+, Positive; –,Negative; NA, not available.
UPA, Pathologic Anatomy Unit, Faculty of Veterinary Medicine, Teramo, Italy; IZSAM, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy; IZSLT, Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Rome, Italy.

Discussion

The present data suggest that *Troglostrongylus* spp. have not been frequently misdiagnosed or confused for *A. abstrusus* in previous cases of lungworm infestations in cats from Italy. *A. abstrusus* was identified microscopically or by molecular techniques in 68 cats examined in studies 1–4, while *T. brevior* was missed in only one of those animals. The positive PCR results for both *A. abstrusus* and *T. brevior* in 1/68 cats (study 2) demonstrates that, while cases of mixed infestations may have been missed in previous studies, the prevalence of mixed infestations is low. The results obtained from postmortem samples also demonstrated a low prevalence of *T. brevior* and suggest that underestimation of the prevalence of *Troglostrongylus* spp. in domestic cats in the past decade is unlikely.

The cause of death was ascribed to *A. abstrusus* in 11/53 (21%) cats at postmortem examination (Table 2). Death occurred mainly in young cats and in the presence of other diseases, such as bacterial infections or other parasitic infestations (Table 2). Histopathology in cat 52 indicates that *A. abstrusus* may also inhabit large bronchi in addition to bronchioles, alveolar ducts and alveoli. The presence of adult *T. brevior* in the bronchi and bronchioles of cat 39 (Fig. 1a, Table 2) and in a bronchiole of cat 49 (Fig. 1b, Table 2)

Table 2
Respiratory lesions and presence or absence of lungworm stages at postmortem examination in cats in Italy (2002–2013).

Cat	Cause of death	Gross lesions	Histological findings			
			L	A	Br	B
UPA						
1	Dilated cardiomyopathy	C, O	Moderate HPA	–	–	–
2	Vaso-vagal shock and uraemia	Ac	Moderate HPA, E, Bc	–	–	–
3	Feline panleucopaenia	CA, O	FP, O, C, Ac	–	–	–
4	Severe hepatic failure	O	Severe HA, O	–	–	–
IZSAM						
5	Phorate poisoning	C	–	–	–	–
6	Phorate poisoning	–	EI	–	–	–
7	Parasitic pneumonia	N, HCA	–	Larvae and eggs	Larvae and eggs	–
8	Parasitic pneumonia, ascarids	N	P	Larvae and eggs	–	–
9	Parasitic pneumonia	Diffuse LC	HPA	Larvae and eggs	–	–
10	Bacterial pneumonia	C, O	O	–	–	–
11	Thoracic effusion	CA, Ac	P	–	–	–
12	Not determined	–	P	Larvae and eggs	–	–
13	Parasitic pneumonia	CCA	GF,C	–	–	–
14	Parasitic pneumonia	Diffuse LC	GIP, HPA	Larvae and eggs	–	–
15	Bacterial and parasitic pneumonia, ascarids	C	P	Larvae and eggs	–	–
16	Bacterial and parasitic pneumonia, ascarids	–		Larvae, eggs and few adults	–	–
17	Renal failure	CA, PC	GIP, HPA	Larvae and eggs	–	–
18	Trauma	LC	GIP, HPA	Larvae and eggs	Larvae and eggs	–
19	Nephritis and hepatitis	O	O, HPA	Larvae	–	–
20	Parasitic pneumonia	–	GF	Larvae and eggs	–	–
21	Parasitic pneumonia	O, LC	GF, P, EP	Larvae and eggs	–	–
22	Metaldehyde poisoning	LC	LCA	Larvae and eggs	–	–
23	Parasitic pneumonia	C	EP, HPA	Adults	–	–
24	Bacterial and parasitic pneumonia	C, O, P	P, C, HPG	Adults	–	–
25	Parasitic pneumonia	LC, P	GF	Adults, larvae, eggs	–	–
26	Trauma	Diffuse P	HPA, HPG	–	–	–
27	Feline infectious peritonitis, feline panleukopenia	CA	–	–	–	Adults
28	Nephritis and hepatitis, ascarids	–	HPA	–	–	–
29	Bacterial pneumonia and pleurisy, nephritis	CCA	PP	Few eggs	–	–
30	Trauma	CA	GF	Larvae and eggs	–	–
31	Parasitic pneumonia	LC	GIP	Adults, larvae and eggs	–	–
32	Parasitic pneumonia	Diffuse P	GIP	Larvae and eggs	–	–
33	Bacterial and parasitic pneumonia	P	GIP	Eggs	–	–
34	Bacterial and parasitic pneumonia	Scattered LC	HPA, HPG	–	–	–
IZSLT						
35	Feline infectious peritonitis	O, HE	HPA	–	–	Few adults
36	Feline panleukopenia	CCA	Severe GIP, HPA	Larvae and eggs	Larvae and eggs	–
37	Feline panleukopenia	CCA	GF	–	–	–
38	Feline panleukopenia	CA, severe PP	Severe GIP	Larvae and eggs	–	–
39	Pyothorax	Severe PPC	Severe GIP, HPA	Larvae and eggs	Adults	Adults
40	Bacterial pleuropneumonia	PP	HPA	Adults	Adults	–
41	Bacterial pleuropneumonia	PP	GF, HPA	–	–	–
42	Parasitic pneumonia	Diffuse LC	GF	–	–	–
43	Trauma	N	GF	–	–	–
44	Pyothorax	–	GF	–	–	–
45	Feline infectious peritonitis	O, HE	GF	–	–	–
46	Bacterial septicaemia	Scattered LC	Severe GIP	Larvae and eggs	–	–
47	Bronchoalveolar carcinoma	Large LC	GF	–	–	–
48	Perioperative complications	Scattered LC	GF, HPA	–	–	–
49	Bacterial pneumonia	Diffuse congestive P	GF	–	Adults	–
50	Feline infectious peritonitis	O, HE	GF	–	–	–
51	Poisoning (unspecified)	Numerous calcified N	GF	–	–	–
52	Bacterial pneumonia	Large HCA	–	Few larvae	Adults	Adults
53	Trauma	O, HE	GF	–	–	–

L, Lung (respiratory) lesions; A, alveoli; Br, bronchioles; B, bronchi; T, trachea; Ac, atelectasis; Bc, catarrhal bronchitis; C, congestion; CA, consolidated areas; CCA, congestion and consolidated areas; E, pulmonary emphysema; EI, eosinophilic infiltrate; EP, eosinophilic pneumonia; FP, fibrinous pneumonia; GF, granulomatous foci; GIP, granulomatous/interstitial pneumonia; HA, haemorrhagic areas; HCA, haemorrhagic and consolidated areas; HPA, hypertrophy and/or hyperplasia of smooth muscle in the media of pulmonary vessels and alveolar ducts; HPG, hyperplasia of peribronchiolar glands; HE, haemorrhagic effusions; LC, lardaceous and consolidated areas; N, nodules; O, pulmonary oedema; P, pneumonia; PC, pleurisy and pericarditis; PP, pleuropneumonia; UPA, Pathologic Anatomy Unit, Faculty of Veterinary Medicine, Teramo, Italy; IZSAM, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy; IZSLT, Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Rome, Italy.

is consistent with previous findings (Brianti et al., 2012). The lack of detection of parasites in a few cats (Table 2) with pathological and/or genetic findings consistent with infestation by *A. abstrusus* and/or *T. brevior* may be explained by the selection of portions of tissues for histological examination. Furthermore, severe lesions were observed in the lungs of cats that were positive by PCR for *A. abstrusus* but negative on histological examination (cats 3, 4 and 28; Table 2).

Two cats with histological findings compatible with the presence of nematodes belonging to the genus *Troglostrongylus* were either PCR negative (cat 38) or PCR positive (cat 35) only to

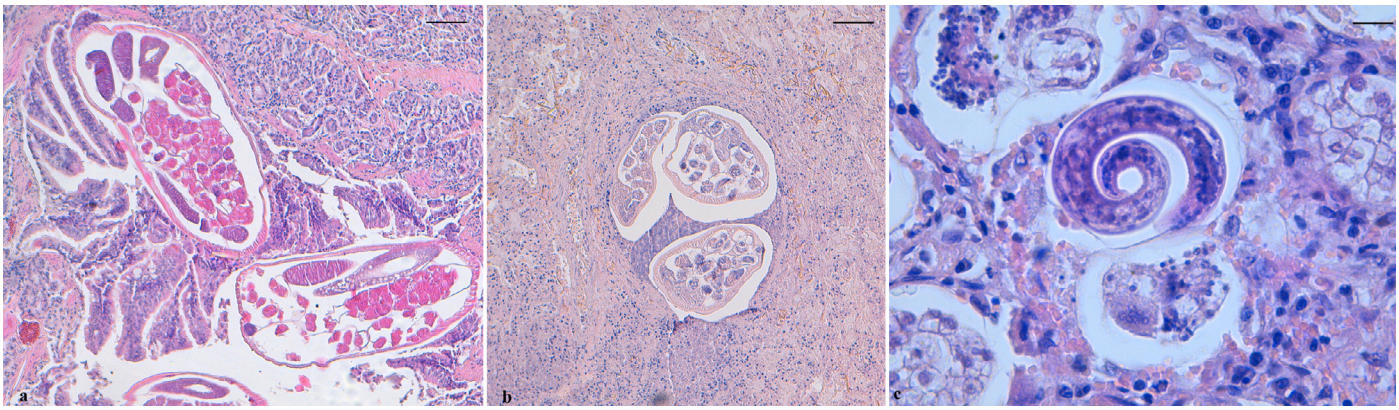

Fig. 1. (a) Adult nematodes in a bronchus of cat 39, which was PCR positive for *Aelurostrongylus abstrusus* and *Troglostrongylus brevior*. Scale bar = 100 μm. (b) Adult nematodes in a bronchiole of cat 49, which was PCR positive for *Troglostrongylus brevior*. Scale bar = 100 μm. (c) Nematode first stage larva in an alveolus of cat 38, which was PCR negative for *Aelurostrongylus abstrusus* and *Troglostrongylus brevior*. Scale bar = 15 μm.

A. abstrusus (Tables 1 and 2). The length and width of the larvae detected in the alveoli of cat 38 (Fig. 1c) were consistent with *T. subcrenatus* (Railliet and Henry, 1913; Brianti et al., 2012). The absence of adult parasites on histological examination of sections of lung from this cat (Table 2) may be because adults of *T. subcrenatus* usually localise in the trachea (Brianti et al., 2012). Adult stages compatible with *Troglostrongylus* spp. (i.e. width ~300–500 μm; Brianti et al., 2012) were also found in the bronchi of cat 35 (Table 2). The negative *T. brevior* PCR result for this cat could be explained by the presence of *T. subcrenatus*. Unfortunately, no DNA sequence is available for *T. subcrenatus* and thus no further genetic confirmation was possible. The paucity of information on the occurrence of *T. subcrenatus* in cats (Fitzsimmons, 1961; Brianti et al., 2012) suggest that this parasite has a limited distribution in domestic hosts.

Infection with *Troglostrongylus* spp. has serious clinical implications in kittens, in which it often causes severe respiratory signs, regardless of the occurrence of secondary pathogens (Brianti et al., 2012, 2013; Traversa and Di Cesare, 2013; Di Cesare et al., 2014a, b). The cause of death in the two cats aged ~5 years in the present study, which were PCR-positive for both *A. abstrusus* and *T. brevior* was pulmonary neoplasia (cat 47) and pyothorax (cat 39), respectively. While the tumour of cat 47 was unlikely to have been caused by lungworms, the pyothorax in cat 39 could have been triggered by the presence of *T. brevior*, even though this parasite usually displays a mild pathogenic potential in adult cats. The cat (~1 year old) with a monospecific infection by *T. brevior* (cat 49) died of bacterial pneumonia (Tables 1, 2), that may have been secondary to the nematode infection. Little information is available on the role of lungworms in causing bacterial infections of the lungs, although concurrent lungworm infection and bacterial pneumonia by *Salmonella* (two cats) and *Escherichia coli* (one cat) has been previously described (Barrs et al., 1999; Foster et al., 2004; Foster and Martin, 2011). The two animals which were perhaps infected by *T. subcrenatus* died of feline infectious peritonitis (cat 35, age not determined) and panleukopenia (cat 38, 2 months), although both had lung lesions consistent with verminous pneumonia.

In addition to two cases from Ibiza, Spain (Jefferies et al., 2010) and one case from Crete (Diakou et al., 2014), *Troglostrongylus* spp. in domestic cats have only been reported from Italy (Paggi, 1959; Brianti et al., 2012, 2013; Di Cesare et al., 2014a, 2014b; Tamponi et al., 2014). Cases of mixed infestations with *A. abstrusus* and *T. brevior* have been reported from central Italy (Abruzzi and Umbria) and southern Italy (Apulia) (Annoscia et al., 2014; Di Cesare et al., 2014a, 2014b). The present results indicate that *A. abstrusus* is the major lungworm species implicated in feline verminous pneumonia in Italy.

Given that the normal hosts of *T. brevior* seem to be wild felids, the limited number of these animals even in their natural habitats may account for the low prevalence of infestation with this lungworm the three subspecies of *Felis* spp. in Italy (*Felis silvestris libyca*, *F. s. silvestris* and *F. s. catus*), have isolated gene pools (Randi et al., 2001; Lecis et al., 2006), but there is potential for cross-breeding between wild and domestic cats (Pierpaoli et al., 2003). Expansion in the geographical distribution of *F. s. silvestris* has been observed in Northern Italy (Lapini, 2006). Wild cats are considered to be the source of *T. brevior* for domestic cats in Italy (Falsone et al., 2014).

Conclusions

The findings of the present study support the major role of *A. abstrusus* in causing respiratory infestations of cats in Italy. Additional epidemiological studies are warranted to evaluate changes in the geographic dispersion of *T. brevior* and other 'wild' metastrongyloids (e.g. *T. subcrenatus*, *Oslerus rostratus*) and their possible impact in the pathology of respiratory parasitoses of domestic cats.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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