Lungworms and gastrointestinal parasites of domestic cats: a European perspective

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A R T I C L E   I N F O

Article history:
Received 17 December 2016
Received in revised form 23 February 2017
Accepted 24 February 2017
Available online xxxx

Keywords:
Cats
Lungworms
Gastrointestinal parasites
Epidemiology
Treatment
Diagnosis
Broadline®

A B S T R A C T

With the exception of Aelurostrongylus abstrusus, feline lungworms have been poorly studied. Information on their distribution is patchy and mostly limited to case reports. In this study, the occurrence of feline lungworms and co-infecting gastrointestinal parasites has been investigated in 12 European countries (i.e. Austria, Belgium, Bulgaria, France, Greece, Hungary, Italy, Portugal, Romania, Spain, Switzerland and the United Kingdom). An average of 10 domestic cats, with regular outdoor access, was sampled each month for 12 months, and freshly passed faeces were collected. Stools were processed using a McMaster assay and a quantitative Baermann-Wetzel method. Animals positive for lungworms and/or gastrointestinal infections were treated with a formulation containing fipronil, (S)-methoprene, eprinomectin, and re-sampled 28 days post-treatment. The association between lungworm infection and risk factors was analysed using statistical medians/means and the efficacy of the treatment against each lungworm species was assessed. Of 1990 cats sampled, 613 (30.8%) were positive for at least one parasite, while 210 (10.6%) were infected by lungworms. The prevalence of lungworm infection varied between the sampled sites, with the highest recorded in Bulgaria (35.8%) and the lowest in Switzerland (0.8%). None of the cats from Austria or the United Kingdom were infected by lungworms. Aelurostrongylus abstrusus was the species most frequently detected (78.1%), followed...
1. Introduction

Nematodes are amongst the most important etiological parasitic agents of gastrointestinal and respiratory alterations in cats (Deplazes et al., 2016; Taylor et al., 2016). It has been estimated that these pathogens affect 20–40% of cats in Europe, with varying prevalence rates, depending upon the pathogen species, the animal population size, the sampling area and the diagnostic procedures (Mircean et al., 2010; Baruztki and Schaper, 2011; Capári et al., 2013; Beugnet et al., 2014; Knaus et al., 2014a; Elsheikha et al., 2016). For instance, studies on lungworms are mostly limited to case reports on clinically affected cats and to a few epidemiological surveys (Traversa et al., 2010). Consequently, the clinical and epidemiological relevance of these nematodes in feline populations has been largely underestimated. In addition, limitations in current diagnostic techniques and inherent difficulties in the morphological identification of species may have hindered progress in this area (Traversa and Di Cesare, 2013; Otranto et al., 2013; Otranto, 2015).

The metastrongyloid Aelurostrongylus abstrusus (Strongylida, Angiostrongyliidae) (Deplazes et al., 2016) and the trichuroid Eucoleus aerophilus (Trichurida, Trichinellidae, syn. Capillaria aerophila) (Deplazes et al., 2016) are considered the most important causes of parasite-induced respiratory infection in felids (Anderson, 2000; Traversa and Di Cesare, 2013). Nonetheless, other lungworms may induce verminous bronchopneumonia. These include species of Troglostrongylus (Strongylida, Crenosomatidae) (Deplazes et al., 2016), of which increasing reports (Jefferies et al., 2010; Brianti et al., 2012, 2014a) have contributed to raise questions about their actual distribution and clinical relevance in veterinary medicine. Indeed, knowledge of the fundamental biology and epidemiology of this parasite genus is limited, most likely due to the morphological similarity between L1s of Troglostrongylus brevior and those of the better-known A. abstrusus (Otranto et al., 2013; Traversa and Di Cesare, 2013). In spite of the different anatomical localizations of the adult parasites (with A. abstrusus in the bronchioles, alveolar ducts and alveoli, and T. brevior in the upper respiratory tract), both species inhabit the lung airways (Gerichter, 1949; Giannelli et al., 2014a), and share the same definitive hosts (i.e. domestic and wild cats, and lynxes) (Gerichter, 1949; Alić et al., 2015; Diakou et al., 2015; Otranto, 2015) and some species of gastropod intermediate hosts (Hobmaier and Hobmaier, 1935; Gerichter, 1949; Giannelli et al., 2014b).

Epidemiological studies of feline lungworms are primarily limited to the Mediterranean area (e.g. Albania, Italy, Spain and Greece) (Jefferies et al., 2010; Knaus et al., 2011; Tamponi et al., 2014; Diakou et al., 2015; Di Cesare et al., 2015a; Giannelli et al., 2015a). Nonetheless, the intricate puzzle regarding their distribution is further complicated by the occurrence of other nematode species, including Oslerus rostratus (Strongylida, Filarioïdidae), Troglostrongylus subcrenatus (Strongylida, Crenosomatidae) and Angiostrongylus chabaudi (Strongylida, Angiostrongylidae) in both domestic and wild cats (Anderson, 2000; Brianti et al., 2012, 2014b; Varcasia et al., 2014; Deplazes et al., 2016; Giannelli et al., 2016a).

Undoubtedly, information on the distribution of these pathogens of felids needs to be updated and extended to geographical areas where the occurrence of these parasites has been rarely investigated. Therefore, in the present study, a detailed analysis of the distribution of lungworm species, focusing on metastrongyloids, has been assessed in cats living in 12 European countries, together with data on co-infecting gastrointestinal parasites.

2. Materials and methods

2.1. Study locations

From March 2015 to February 2016, 15 veterinary academic institutions contributed to a multicentric field study: one each in Austria (Vienna), Belgium (Liege), Bulgaria (Stara Zagora), Greece (Thessaloniki), Hungary (Budapest), Romania (Cluj-Napoca), Spain (Madrid), Switzerland (Zurich), and the United Kingdom (Cambridge); three each in Italy (Bari, Messina, Sassari) and in Portugal (northern, central and southern areas, including Lisbon). In addition, in France (Lyon), samples were collected from June 2015 to May 2016. The same procedures were applied across all institutions.

2.2. Inclusion criteria

Approximately 10 cats referred to the Faculty Hospital at each academic institution or to local practitioners were sampled each month. Informed consent and agreement were obtained from the owners of the cats before enrolment. The examination of cats was conducted with regards to animal welfare and the protocol was approved by each local ethics committee. All cats had a history of regular outdoor access (i.e. they were exposed to external environments) and were included in the study only if in good general health condition and if not treated with any anthelmintic drugs at least over the month prior to sample collection. At the first screening, a complete anamnesis, which included animal age, gender, weight class (i.e. less or more than 2.5 kg), geo-referenced locality, lifestyle (i.e. owned, stray or cattery) and occurrence of respiratory alterations were recorded, together with freshly passed faeces (6–10 g), which were collected and processed according to standardized procedures (see Section 2.3).

2.3. Faecal examinations

At each institution, faecal samples were analysed using a standard McMaster technique and a quantitative Baermann-Wetzel method, for the detection of parasites shedding eggs/oocysts and metastrongyloid larvae, respectively.

2.3.1. McMaster assay

Two g of faeces, either fresh or refrigerated at 4 °C, were processed within 24 h. Samples were weighed into a mortar and homogenized with 28 ml of zinc sulphate solution (ZnSO4, specific gravity = 1.2), until a final volume of 30 ml was reached. The suspension was mixed and filtered through double-layered gauze into a clean beaker cup. McMaster chambers were filled with the faecal suspension using a pipette. After 1–2 min, floating parasitic elements were counted, the number of eggs/oocysts per gram (EPG/OPG) was calculated, and the parasite identified at group
level (Sously, 1965; Euzey, 1981; Zajac, 1994). At the Institute of Parasitology (University of Zurich, Switzerland), the McMaster technique was performed using an oversaturated sodium chloride solution (NaCl, specific gravity = 1.195–1.205).

2.3.2. Baermann-Wetzel method

This assay was performed as described in Giannelli et al. (2015a). Briefly, 1 g of faeces was placed on double-layered gauze, secured with a wire; the sample was settled into a Baermann funnel, filled with 50 ml of tap water, and examined after 24 h. The solution was poured into a tube and centrifuged at 600 g for 5 min; the supernatant was removed and the sediment placed on a microscope slide for examination. Larvae were identified at the genus level, and counted, thus assessing the number of L1s per gram (LPG).

2.4. Treatment and follow-ups

Animals positive for gastrointestinal helminths and/or lungworms were treated by practitioners with a topical application of the combination of fipronil ((S)-methoprene/eprinomectin/prazi quantel (Broadline®, Merial, France). The product, administered according to the manufacturer’s instructions, was delivered directly onto the skin in the midline of the neck, between the base of the skull and the shoulder blades in a single spot. Cats weighing 0.8–2.5 kg received one 0.3 ml application, while those of 2.5–7.5 kg body weight received one 0.9 ml application, as indicated in the label dose. Animals were observed for health problems (i.e. skin reactions, drooling or neurological signs) and adverse events were recorded.

Cats scoring positive for lungworm L1s/eggs were followed up 28 days after the treatment. Their faeces were collected and examined as previously described. In the case of further occurrence of lungworm eggs/L1s, an additional treatment was administered and the animal was again examined for the presence of parasites 15 ± 2 days after the second treatment.

2.5. Parasite identification

Following the primary identifications of parasites at each site (see Section 2.3), the sediment of the Baermann-Wetzel test and the supernatant of the flotation solutions, collected from cats positive for lungworms, were transferred to the University of Bari, Italy, where lungworms were morphologically and molecularly identified at species level. Each sample was divided into two aliquots of 1 ml each and preserved in 70% ethanol or 10% formalin, respectively.

2.5.1. Morphological examination and identification of larvae

Larvae and eggs stored in formalin were photographed, measured by an optical microscope (Leica®, DMLB2), analysed with LAS AF 4.1 software, and morphologically identified to species level according to key descriptions (Gerichter, 1949; Anderson, 2000; Traversa et al., 2011; Brianti et al., 2012, 2014b; Giannelli et al., 2014b). In the case of metastrongyloids, measurements of larval body length, position of the oral opening and tail morphology (i.e. occurrence of dorsal kinks, depth of incisures and features of terminal endings) were the main features considered and compared with the larval length intervals reported by Gerichter (1949), commonly used for A. abstrusus, T. brevior and O. rostratus differentiation (i.e. 360–390 μm, 300–310 μm and 300–320 μm, respectively).

2.5.2. Molecular confirmation of lungworm larvae

Genomic DNA was extracted from larvae isolated from the Baermann sediment and preserved in 70% ethanol, using a commercial kit (QIAamp® Fast DNA Stool Mini Kit, Qiagen, GmbH, Hilden, Germany) in accordance with the manufacturer’s instructions. A duplex-PCR targeting the ribosomal internal transcribed spacer 2 region (ITS 2) was used for the discrimination of A. abstrusus and/or T. brevior larvae (Annoscia et al., 2014). Conversely, in samples scoring negative for the previous reaction and/or that contained specimens not identifiable according to their morphology, the 18S rRNA gene was amplified, according to the protocol by Patterson-Kane et al. (2009), and sequenced. Amplicons were resolved in ethidium bromide-stained agarose (Gellyphor, Vilnius, Lithuania), as a molecular marker. Gels were photographed using Gel Doc 2000 (Bio-Rad, Hercules, CA, USA). Sequences were determined from both strands, using the same primers individually as for the PCR, and the electropherograms were verified manually. The nucleotide sequence was conceptually translated into the amino acid sequence, using the invertebrate mitochondrial code MEGA5 software. Sequences were compared with those available in the GenBank® database by using the Basic Local Alignment Search Tool (BLAST).

2.6. Statistical analysis

Prevalence values were calculated as the proportion of positive animals to the total number of examined animals, whereas the relative prevalence of each parasite species was calculated as the proportion of cats infected by a given nematode species within the total number of positive results. The association between lungworm prevalence and cat data (i.e. location, gender, age, lifestyle and co-infection with other parasites) was investigated using a χ² test. The animals were categorized into four age classes (i.e. <6, 6–12, 12–24 and >24 months). The seasonality of lungworm diagnosis was evaluated according to the climatic region (Mediterranean area: Italy, Spain, Portugal and Greece; continental area: Bulgaria, France, Switzerland, Belgium, Hungary and Romania).

2.7. Evaluation of the treatment efficacy

The geometric means for each species of lungworm LPG/EPG were calculated after transformation of the data (count +1) into the natural logarithm. The efficacy of the treatment was assessed as a percentage, using the formula: efficacy (%) = 100[(C−T)/C], where C was the geometric mean of larvae before the treatment and T was the geometric mean following the first administration of the product. The efficacy of the treatment was estimated by an ANOVA, followed by a Tukey test for pairwise comparisons. All analyses were two-sided and the significance level was set at P < 0.05. The software used was SPSS for Windows, version 13.0.

2.8. Data accessibility

Raw data on animal location, age, co-infections, and positivity for lungworms are available on Mendeley data (Doi: http://dx.doi.org/10.17632/y6krk94vdp.1).

3. Results

3.1. Sampled population

A total of 1990 domestic cats (i.e. 935 males, 1043 females, 12 gender not reported), recruited from 424 different localities, were sampled (Table 1). Animals were aged from 30 days to 24 years (mean 3.2 years, median 2.2 months). Of these, 322 (16.2%) were less than 6 months old, 462 (23.2%) between 6 months and 1 year old, 372 (18.7%) between 1 and 2 years old, and 815 (41.0%) were...
over 2 years old. Data on the age of the remaining 19 cats was unavailable. Most of the animals (1448, 72.8%) were owned (i.e. they lived indoors but had daily outdoor access), whereas 301 (15.1%) and 238 (12.0%) cats were previously strays or housed in catteries, respectively. No data was available for the remaining three animals.

A total of 613 cats (30.8%, 95% Confidence Interval (CI): 29–33%) were infected by at least one parasitic pathogen; ascarids were the most prevalent parasites (16.5%, 95% CI: 15–18%), followed by lungworms (10.6%, 95% CI: 9–12%), coccidia (6.5%, 95% CI: 5–8%) and hookworms (4.5%, 95% CI: 4–5%) (Table 1).

### 3.2. Distribution of cats infected by lungworms

Lungworms were recorded in 88/424 (20.8%) localities from all the countries examined, with the exception of Austria and the United Kingdom. The prevalence of the infections varied among the sampled sites, with the highest recorded in Bulgaria (35.8%) and the lowest in Switzerland (0.8%) ($\chi^2 = 188.64; P < 0.001$). Amongst the lungworm-infected animals, 110 (52.4%) were co-infected by gastrointestinal parasites, with two (n = 72; 65.5%), three (n = 28; 25.5%) or four (n = 10; 9.1%) parasite species detected simultaneously (Table 2). Animals positive for lungworms (n = 210; 10.6%) were aged from 1 month to 15 years (arithmetic mean 2.1 years, median 1.5 years), with different prevalences according the risk factors assessed (Table 3). A significantly higher prevalence was found in cats younger than 2 years ($\chi^2 = 14.562; P = 0.002$) or in those co-infected by other gastrointestinal parasites ($\chi^2 = 17.024; P < 0.001$) (Table 3).

### 3.3. Lungworm species

Four lungworm species were detected by morphological and/or molecular analyses. In particular, 178 cats (84.8%) were positive for a single lungworm species, and 32 (15.2%) were infected by more than one species (Table 4). *Aelurostrongylus abstrusus* was the species most frequently diagnosed (n = 164; 78.1%; 95% CI: 72–83%), followed by *T. brevior* (n = 41; 19.5%, 95% CI: 15–25%) and *E. aerophilus* (n = 31; 14.8%; 95% CI: 11–20%) and *O. rostratus* (n = 8; 3.8%; 95% CI: 2–7%) (Table 4). The identification of 172 samples was assessed by studying the morphology of L1s (see Section 3.6), and was confirmed by the duplex-PCR. Conversely, 30 samples (i.e. 10 from Greece, six from Italy, four from Romania, four from Spain, three from Portugal, two from Hungary, one from France) contained unidentifiable L1s. Their identification was assessed by amplifying the 18S rRNA gene and sequences showed 100% nucleotide identity with those of the same species deposited in GenBank® (*A. abstrusus*, AJ920366 and *T. brevior*, JX290562). No sequences were obtained for the eight samples positive for *O. rostratus*.

### 3.4. Evaluation of larval/egg shedding, cat age and diagnosis seasonality

The prevalence of lungworm infections and their distribution are reported in Table 4 according to the sampled areas. The infection intensity (expressed as the number of LPG/EPG) varied among the lungworm species, with a mean of 8094.9 *A. abstrusus* LPG, 742.5 *T. brevior* LPG, 237.5 *O. rostratus* LPG and 143 *E. aerophilus* LPG. Cats from Portugal, Italy (Bari), Hungary and Bulgaria shed the highest numbers of *A. abstrusus* (mean LPG = 90,492.9, range 50–620,000), *T. brevior* (mean LPG = 1601, range 60–4000), *O. rostratus* (mean LPG = 441 LPG, range 102–986), and *E. aerophilus* EPG (mean EPG = 169.2, range 100–400), respectively. Infections by *A. abstrusus* and *E. aerophilus* were most frequently diagnosed in animals aged from 6 months to 2 years, whereas those by *T. brevior* in cats younger than 1 year of age (Fig. 2); however, these cor-
relations were not statistically significant. Six out of the eight O. rostratus-positive cats were older than 2 years. Lungworm infections were diagnosed throughout the study period, with the majority of cases reported in the spring (74/499, 14.8%) and winter of 2015 (51/422; 12.1%) ($\chi^2 = 18.132; P < 0.01$). The largest number of cats infected by metastrongyloids was recorded during spring (41%) or winter (36.6%), in Mediterranean or continental areas, respectively.

3.5. Clinical observations

Respiratory alterations (i.e. cough, dyspnoea, and nasal discharge) were reported in 14 (6.7%) cats positive for lungworms. The age of clinically affected animals was 7 months (mean 11.6 months, range 3 months to 3 years). Five of these cats were infected by A. abstrusus or by T. brevior, respectively, one was co-infected with both species and three were infected by A. abstrusus and E. aerophilus, with mean numbers of 772.4 (range 19–187,200 LPG) and 1345.4 (range 63–4000 LPG) for A. abstrusus and T. brevior LPG, respectively, and 50 E. aerophilus EPG. Three cats (mean age 5.5 months) infected by A. abstrusus from Lisbon (Portugal) and one co-infected by E. aerophilus from Cluj Napoca (Romania) died over the course of the study; these shed the largest number of A. abstrusus LPG recorded (mean LPG = 358,925; range = 137,500–620,000).

3.6. Morphological identification of larvae

From 180 samples which were morphologically and molecularly identified, more than 3000 metastrongyloid larvae stored in
formalin were measured and, of those, 1250 were processed for detailed morphological identification.

### 3.6.1. Body length of larvae

Those specimens included 967 *A. abstrusus*, 264 *T. brevior*, and 19 *O. rostratus* L1s, and their mean length was 364.1 ± 35.5 μm (range 210.4–495.1 μm), 304.1 ± 27.9 μm (range 203.2–382.2 μm) and 332.1 ± 35.9 μm (range 216.6–366.8 μm), respectively. The body length of *A. abstrusus* L1s displayed some difference according to sampling countries (ANOVA, $F = 25.233$, $P < 0.001$) (Table 5). When compared with the larval length reference intervals used within the expected range (Fig. 3). In particular, 407 (42.1%) *A. abstrusus* L1s were located within the range 310–360 μm, whereas 111 (42.0%) *T. brevior* and 18 (94.7%) *O. rostratus* L1s exceeded the expected upper length limit (Fig. 3).

### 3.6.2. Morphological features of larvae

The morphology of the anterior extremity and the position of the oral opening of L1s were consistent with the species of metasstrongyloid examined, whereas slight variations in the shape of the caudal extremity were detected within the same species. In particular, the tail shape was variable and differences in the morphology of dorsal kinks, incisure depths and terminal endings were recorded. In brief, L1s of *A. abstrusus* featured a slender anterior extremity, with a short and terminal oral opening leading into a narrow vestibule; the tail was bent into an S-shape, with a visible dorsal kink, distinct deep dorsal and ventral incisures, and a terminal knob-like extremity (Fig. 4A). Larvae of *T. brevior* were characterised by a clear and pointed anterior extremity, with a sub-terminal oral opening; the tail featured a deep dorsal incision, which divided the extremity into two appendices (a shallow ventral one and a slender dorsal one). The S-shape of the posterior extremity was not obvious, and the ending was straight and gradually tapered (Fig. 4B). Finally, *O. rostratus* L1s were characterized by a rounded head, with a central oral opening and a cylindrical buccal capsule; the tail was slightly undulated, with a deep ventral notch and a shallow dorsal one. The notch ended in a minute spine (Fig. 4C).

### 3.7. Evaluation of treatment efficacy

The treatment efficacy against lungworms was assessed in 198 cats. Twelve cats did not complete the assessment. At enrolment, 145 cats were co-infected with 2 or more gastrointestinal parasites, whereas 53 cats were affected by only one parasite species. Within the 30 cats that completed the treatment, 10 cats were co-infected with more than one parasite species. Twelve cats did not complete the assessment.

---

**Table 3**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Class</th>
<th>Number tested</th>
<th>Positive (%)</th>
<th>Chi-square</th>
<th>Significance</th>
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<td>Age (months)</td>
<td>&lt;6</td>
<td>322</td>
<td>35 (10.9)</td>
<td>14.562</td>
<td>$P = 0.002$</td>
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<td></td>
<td>6–12</td>
<td>462</td>
<td>64 (13.9)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>12–24</td>
<td>372</td>
<td>47 (12.6)</td>
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</tr>
<tr>
<td></td>
<td>&gt;24</td>
<td>815</td>
<td>62 (7.6)</td>
<td></td>
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<tr>
<td>Climatic area</td>
<td>Continental</td>
<td>1227</td>
<td>137 (11.2)</td>
<td>1.273</td>
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<tr>
<td></td>
<td>Mediterranean</td>
<td>763</td>
<td>73 (9.6)</td>
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<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>1043</td>
<td>114 (10.9)</td>
<td>0.309</td>
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<tr>
<td></td>
<td>Male</td>
<td>935</td>
<td>95 (10.2)</td>
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<tr>
<td>Other infections</td>
<td>No</td>
<td>1525</td>
<td>137 (9.0)</td>
<td>17.024</td>
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<td>Yes</td>
<td>465</td>
<td>73 (15.7)</td>
<td></td>
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</tr>
<tr>
<td>Lifestyle</td>
<td>Owned</td>
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<td>159 (11.0)</td>
<td>2.584</td>
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<td></td>
<td>Stray</td>
<td>301</td>
<td>33 (11.0)</td>
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<td></td>
<td>Cattery</td>
<td>238</td>
<td>18 (7.6)</td>
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</tbody>
</table>

ns, not significant.

$^t$ Data was not available for 19 cats.

---

**Table 4**

<table>
<thead>
<tr>
<th>Sampling areas</th>
<th>(animals positive for lungworms, %)</th>
<th>Lungworm species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Aelurostrongylus abstrusus</em></td>
</tr>
<tr>
<td>Belgium (n = 2/108, 1.9%)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Bulgaria (n = 43/120, 35.8%)</td>
<td></td>
<td>20 + 7$^a$ + 4$^b$ + 2$^d$</td>
</tr>
<tr>
<td>France (n = 7/92, 7.6%)</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Greece (n = 13/118, 11%)</td>
<td></td>
<td>8 + 2$^a$</td>
</tr>
<tr>
<td>Hungary (n = 30/120, 25%)</td>
<td></td>
<td>27</td>
</tr>
<tr>
<td>Italy, Bari (n = 20/120, 16.7%)</td>
<td></td>
<td>2 + 4$^e$</td>
</tr>
<tr>
<td>Italy, Sassari (n = 16/138, 11.6%)</td>
<td></td>
<td>14 + 2$^f$</td>
</tr>
<tr>
<td>Italy, Messina (n = 26/170, 15.3%)</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Portugal, Centre and South (n = 2/120, 1.7%)</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Portugal, Lisbon (n = 14/120, 11.7%)</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Portugal, North (n = 3/120, 2.5%)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Romania (n = 20/120, 16.7%)</td>
<td></td>
<td>12 + 5$^f$</td>
</tr>
<tr>
<td>Spain (n = 13/200, 6.5%)</td>
<td></td>
<td>5 + 5$^f$</td>
</tr>
<tr>
<td>Switzerland (n = 1/124, 0.8%)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>(N = 210/1990, 10.6%)</td>
<td>164</td>
</tr>
</tbody>
</table>

$^a$ *Aelurostrongylus abstrusus* and *E. aerophilus*.
$^b$ *Aelurostrongylus abstrusus* and *T. brevior*.
$^c$ *Troglostrongylus brevior* and *E. aerophilus*.
$^d$ *Aelurostrongylus abstrusus*, *T. brevior* and *E. aerophilus*.
cats shed a mean of 508.7 LPG, 742.5 LPG, and 237.5 LPG of *A. abstrusus*, *T. brevior*, and *O. rostratus*, respectively, and a mean of 153.3 EPG of *E. aerophilus*. All cats tested negative for lungworm larvae after a single treatment, with the exception of three animals from Sassari (Italy), one from Sicily (Italy), and one from France, which were positive for *A. abstrusus*. The overall efficacy of Broad-line was 99% for *A. abstrusus* and 100% for *T. brevior*, *O. rostratus* and *E. aerophilus*, respectively (*P < 0.05*). Clinical respiratory signs subsided in all treated animals. No adverse reactions related to the treatment were observed. The treatment efficacy in animals co-infected by ascarids (21 cats), hookworms, *Strongyloides* sp. (11 cats), *Taenia* spp. (eight cats), *Dipylidium caninum* (two cats), *Hymenolepis diminuta* and *Joyeuxiella pasqualei* (one cat) was 100% (*P < 0.05*).

### 4. Discussion

Data from the present study provides a comprehensive picture of the diagnosis, epidemiology and treatment of feline lungworms in Europe, as well as of the occurrence of co-infections by other gastrointestinal parasites. Indeed, whilst indicating that the prevalence and composition of the endoparasitic fauna of domestic cats is extremely diversified, results also point out that ascarids are the most prevalent parasitic nematodes, as shown in previous reports (Beugnet et al., 2014; Knaus et al., 2014a; Giannelli et al., 2015a; Takeuchi-Storm et al., 2015). The cosmopolitan distribution of ascarids (mainly *Toxocara cati*) is most likely linked to their biology and ecology (e.g. the extreme resistance of eggs to adverse climatic conditions, lactogenic transmission), which drastically increases

---

**Table 5**

Analysis of lungworm L1 length (μm) according to species and sampling area.

<table>
<thead>
<tr>
<th>Lungworm species</th>
<th>Sampling area</th>
<th>Specimens measured (n)</th>
<th>Mean length ± S.D.</th>
<th>95% CI for the mean</th>
<th>MIN-MAX length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td><em>Aelurostrongylus abstrusus</em></td>
<td>Italy, Bari</td>
<td>16</td>
<td>364.7 ± 32.71</td>
<td>347.28</td>
<td>382.14</td>
</tr>
<tr>
<td></td>
<td>Portugal, Lisbon</td>
<td>109</td>
<td>397.3 ± 32.72</td>
<td>381.58</td>
<td>394.00</td>
</tr>
<tr>
<td></td>
<td>Romania</td>
<td>31</td>
<td>386.8 ± 30.04</td>
<td>381.93</td>
<td>391.14</td>
</tr>
<tr>
<td></td>
<td>Bulgaria</td>
<td>377</td>
<td>365.5 ± 37.22</td>
<td>361.08</td>
<td>369.99</td>
</tr>
<tr>
<td></td>
<td>Italy, Sassari</td>
<td>166</td>
<td>350.8 ± 15.68</td>
<td>347.53</td>
<td>354.16</td>
</tr>
<tr>
<td></td>
<td>Italy, Messina</td>
<td>144</td>
<td>381.8 ± 37.22</td>
<td>371.37</td>
<td>392.31</td>
</tr>
<tr>
<td></td>
<td>Hungary</td>
<td>51</td>
<td>315.4 ± 28.28</td>
<td>306.49</td>
<td>316.00</td>
</tr>
<tr>
<td></td>
<td>Spain</td>
<td>66</td>
<td>373.5 ± 24.86</td>
<td>352.35</td>
<td>359.34</td>
</tr>
<tr>
<td></td>
<td>France</td>
<td>7</td>
<td>363.9 ± 35.37</td>
<td>361.69</td>
<td>366.15</td>
</tr>
</tbody>
</table>

| *Troglostrongylus brevior* | Italy, Bari | 103                    | 309.8 ± 35.07       | 303.01             | 316.71          | 203.2–382.2     |
|                         | Italy, Sassari| 6                     | 318.8 ± 25.98       | 312.96             | 324.76          | 220.8–356.7     |
|                         | Italy, Messina| 21                    | 311.2 ± 12.71       | 305.49             | 317.07          | 276.9–340.5     |
|                         | Bulgaria      | 117                    | 296.4 ± 20.86       | 292.58             | 300.22          | 242.9–352.7     |
|                         | Spain         | 17                     | 298.7 ± 23.99       | 296.56             | 312.25          | 256.7–369.3     |
|                         | Total         | 264                    | 304.0 ± 27.87       | 300.69             | 307.45          | 203.2–382.2     |

| *Oslerus rostratus* | Italy, Bari | 7                      | 353.2 ± 9.93        | 346.87             | 358.48          | 336.3–366.8     |
|                     | Spain        | 12                     | 333.1 ± 35.94       | 315.78             | 350.43          | 216.6–366.8     |

Each letter (a–h) corresponds to the same significant difference.

CI, confidence interval.

Fig. 3. Feline lungworm infections diagnosed during this study (scale bars = 100 μm). L1s of *Aelurostrongylus abstrusus* (A), *Troglostrongylus brevior* (B), and *Oslerus rostratus* (C), with magnifications of the anterior (Aa, Ba, Ca) and posterior extremities (Ab, Bb, Cb). Eggs of *Eucoleus aerophilus* (D), with particulars of the polar plug (Da) and of the net-like surface of the egg (Db).

Fig. 4. Range of length (μm) of metastrongyloid L1s according to the species examined. The majority of *Aelurostrongylus abstrusus* (squares), *Troglostrongylus brevior* (triangles), and *Oslerus rostratus* (circles) specimens were within the range 310–360 μm (grey shaded region).
the circulation of this nematode amongst susceptible hosts. Surprisingly, lungworms were the second most frequent group of nematodes diagnosed in the study population, and in half of the cases they concurred with other gastrointestinal parasites including tapeworms, hookworms and coccidia, demonstrating a high level of parasitic co-infection in cats. The simultaneous detection of lungworms together with ascarids, *Joyeuxiella pasqualei* or *Taenia* spp. (Table 1) suggests that infected cats preyed on rodents, which may act as reservoirs for all these pathogens and therefore as a source of infection (Gerichter, 1949; Anderson, 2000; Reperant et al., 2009; Waap et al., 2014; Schuster et al., 2016). Indeed, parasitic hosts play a relevant, but poorly studied, epidemiological role in the distribution of these parasites. Similarly, the finding of *Hymenolepis diminuta* in cats could be associated with the consumption of infected rodents harbouring mature proglottids (Owen, 2005; Schuster et al., 2016).

Based on the results reported herein, at least one out of 10 cats in this study is at risk of lungworm infection in European countries, thus suggesting that these pathogens are more widespread than previously thought (Payo-Puente et al., 2008; Traversa et al., 2010; Beugnet et al., 2014). As previously indicated for *Angiostrongylus vasorum* of canids (Morgan et al., 2009), the geographical distribution of feline lungworms is patchy but stable in endemic spots, as the infection has been consistently detected in the same sampling sites, even years after its first description. Although literature reports are often not comparable due to inconsistent sampling procedures, the number of positive cats recorded in Lisbon (i.e. 11.7%) is similar to that recorded in the same locality from June 2009 to December 2011 (i.e. 12–12.4%) (Nabais et al., 2014; Waap et al., 2014). Conversely, when prevalence data is assessed on a wider scale (e.g. at the regional or national level), comparisons are probably baseless. This explains, for instance, the difference in infection rates detected among the three Italian regions screened (i.e. Apulia, Sardinia and Sicily) both in the present study and previously (Tamponi et al., 2014; Giannelli et al., 2015a), or those assessed in Greece, Hungary, Romania and Spain in previous surveys (Miró et al., 2004; Capári et al., 2013; Mircean et al., 2010; Diakou et al., 2015). Based on these results, feline lungworms cannot be considered as emerging pathogens, as limited data is available to draw conclusions on their changing distribution. The prevalence of the infection and number of LPG/EPG detected vary amongst the cat populations sampled and the lungworm species. Whether this is associated with climatic and ecological factors affecting parasite development and/or of their intermediate/paratenic hosts, or to regular anthelminthic treatment of cats, is yet to be determined. However, a recent survey on parasitises of domestic cats has demonstrated that the administration of regular anthelminthic treatments drastically reduces the risk of *T. cati* infection (Beugnet et al., 2014). Based on this knowledge, we speculate that owned cats living in Bulgaria, Hungary, Italy, Romania or Portugal were treated less frequently than those from other countries, where only a few individuals were positive for lungworms (e.g. Switzerland and Belgium) (Matos et al., 2015). In the meantime, the lack of detection of lungworm-positive cats in Austria and in the United Kingdom suggests that the prevalence of feline metostrongylids in those areas is low, as indicated by a single report of *A. abstrusus* in a kitten from Liverpool (Philby et al., 2014). To the best of the authors’ knowledge, this is the first study assessing the occurrence of feline lungworms in Belgium, France and Switzerland, from where only isolated case reports were previously available (Traversa et al., 2010).

The design of the study did not allow us to determine when the cats had become infected by the parasites; however, the infection was more frequently detected during winter and spring. Although this data prevents us from drawing definitive conclusions, a similar finding was recorded for *A. abstrusus* in Germany (Taubert et al., 2009; Barutzki and Schaper, 2011). Due to the limited knowledge of the epidemiology of the infection in the intermediate and parasitic hosts, the existence of seasonality in feline lungworm infection is difficult to assess. In contrast, whilst analysing the seasonal trend of *A. vasorum* diagnosis in dogs from England, Wales and Scotland during the period 2005–2008, Morgan and colleagues (2010) concluded that the peak of canine angiostrongylosis cases in winter and spring was related to an increase in infected gastropod numbers over the previous months. One might hypothesize that the dynamic of metostrongylid infections in cats ensues from periodic variations associated with the biology of the gastropod intermediate hosts (López et al., 2005; Jezewski et al., 2013; Di Cesare et al., 2013; Giannelli et al., 2014b); however, the interactions between snails and paratenic hosts should also be the subject of further investigations. Indeed, a number of other factors, such as the contamination of the environment with infective L3 s released via the gastropod mucus and the “intermediésis” (Giannelli et al., 2015b; Colella et al., 2015) should be taken into account when considering the possibility that a definitive host comes into contact with the parasite.

The detection of up to four respiratory nematode species in cats indicates that polyspecific infections are likely to occur in felids. This is not surprising, considering the number of broncopulmonary nematodes of sheep and goats (i.e. *Cylicocellus ocreatus*, *Muellerius capillaris*, *Protostrongylus rufescens* and *Dictyocaulus filaria*) (Anderson, 2000; Alasaad et al., 2009), or the cardiopulmonary nematodes of canids, which include a main species (i.e. *A. vasorum*) plus others, characterised by a more limited geographical distribution (i.e. *Crenosoma vulpis*, *Osterus osteri*, *Filarioidea hirthi*, *Eucoleus boehmi*, and *E. aerophilus*) (McCarty and Morgan, 2009; Traversa et al., 2010; Latrofa et al., 2015; Colella et al., 2016; Tolnai et al., 2015; Hodžić et al., 2016).

A similar scenario occurs in felids, with *A. abstrusus* representing the species more frequently diagnosed, probably due to its wide host range (Giannelli et al., 2016a) and its cosmopolitan distribution; this, outside Europe, includes Africa (Di Cesare et al., 2016), the Americas (Fiorello et al., 2006; Gerdin et al., 2011), Australia (Foster et al., 2004), the Middle East (Abu-Madi et al., 2007) China (Yang and Liang, 2015) and the Russian Far East (González et al., 2007). The diagnosis of this lungworm species in each category of cats analysed in this study indicates that feline aelurostrongylosis threatens almost all cats with access to outdoor environments, irrespective of their age, gender or geographical location. This is in agreement with previous studies (Beugnet et al., 2014; Giannelli et al., 2015a), but in contrast to others, in which adult (Mircean et al., 2010; Capári et al., 2013; Knaus et al., 2014a) or young (Traversa et al., 2008) animals more frequently scored positive. Theoretically, both age categories are at risk for the infection. The immune response of cats towards *A. abstrusus* infection is heterogeneous and unpredictable (Schnyder et al., 2014) and although experimental studies showed that the repeated administration of small numbers of L3s may prevent clinical signs and larval excretion (Hamilton, 1969), the paucity of data available on this topic, under natural conditions, does not fully clarify whether infected animals are protected against subsequent infections.

According to the results of the present survey, *T. brevior* is the second most frequently detected lungworm species of cats. Its presence has been confirmed in islands (i.e. Ibiza, Sicily, Sardinia and Crete) and continental areas (Italy, Greece, Bosnia and Herzegovina) of the Mediterranean basin (Traversa and Di Cesare, 2013; Brianti et al., 2014a; Allé et al., 2015; Diakou et al., 2015), and documented, for the first time, in domestic cats from the Balkans. Of note, the finding of this nematode species in Bulgaria supports previous data on the occurrence of this parasite in wild felids from the same geographical area (Jančev and Genov, 1978).
The occurrence of lungworm infections in wild felids provides interesting arguments for discussion of the alleged increases in prevalence of *T. brevior* across Europe, an event epidemiologically associated with a spill-over effect. This phenomenon occurs when a reservoir population, affected by high pathogen prevalence, interacts with a susceptible one (Power and Mitchell, 2004). In the case of *T. brevior*, this occurrence cannot be totally ruled out, considering wild and domestic cats may share habitats (Randi et al., 2001; Falsone et al., 2014). Notably, the differentiation of *T. brevior* infection in most of the cats younger than 1 year of age further indicates that a direct transmission from the queen to preweaned kittens may occur (Brianti et al., 2013).

*Eucoleus aerophilus*, a respiratory nematode widespread across Europe, has been previously detected in cats from Belgium, Bulgaria, Greece, Italy, Portugal and Romania (see also, Mircean et al., 2010; Traversa et al., 2010; Capári et al., 2013; Knaus et al., 2014a; Di Cesare et al., 2015b). The simultaneous diagnosis of *A. abstrusus* in more than half of the cats positive for this capillarid species indicates that both pathogens may share the same transmission pathway, probably via paratenic hosts (i.e. birds, small rodents and insectivores) which previously fed on gastropods and earthworms (Woods et al., 2003). In addition, data demonstrated that cats are also infected by other capillarid nematodes, whose eggs should be morphologically and molecularly differentiated to achieve a correct diagnosis. Indeed, the differentiation between nematodes of the genera *Eucoleus* and *Trichuris* (e.g. *Trichuris felis*, *Trichuris campanula* and *Trichuris serrata*) should be considered when capillarid eggs are detected (Traversa et al., 2011; Ketzis et al., 2015a,b). However, the possibility that these eggs represented pseudoparasites in the cat faeces cannot be ruled out.

*Oderus rostratus* has been diagnosed in cats from Italy, Spain, Hungary and France, besides its previous detection in Israel (Gerichter, 1949), Sri Lanka (Seneviratna, 1955), the United States (Ash, 1970), Spain (Juste et al., 1992; Millán and Casanova, 2009), and Italy (Brianti et al., 2014b; Varcasia et al., 2015). Scant data on the biology, ecology and clinical features of *O. rostratus* (Seneviratna, 1959) currently impair a thorough assessment of its distribution and the low prevalence of the feline oslerosis in Europe could also be associated with difficulties in diagnosis. Indeed, L1s are shed in the environment intermittently, and for a limited time period (Brianti et al., 2014b); in addition, when adult *O. rostratus* settle in the peri-bronchial tissues, encapsulated by a strong fibrous reaction (i.e. the so-called “regressive stage” of the infection), the production of larvae may cease (Brianti et al., 2014b). It should be considered that, due to the scarce information available on the morphology of *O. rostratus* larvae (which are similar to *A. abstrusus* and *T. brevior* L1s), its correct identification is challenging.

Approximately half of metacontrarylloid larvae analysed in this study were outside their reference length range (i.e. 360–390 µm, 300–310 µm, 300–320 µm, for *A. abstrusus*, *T. brevior* and *O. rostratus*, respectively) (Gerichter, 1949). This finding indicates that the differentiation of feline metacontrarylloids cannot be exclusively based on the evaluation of their length, but requires an appreciation of the shape of anterior and posterior extremities (Gerichter, 1949; Brianti et al., 2014a; Giannelli et al., 2014b). The diagnosis of lungworm infection is, however, essential for planning appropriate therapeutic protocols and preventative measures (Gerardin et al., 2011; Elsheikha et al., 2016). Although most animals did not display clinical signs, some presented with recurrent but transitory signs, particularly cough, dyspnoea and nasal discharge. Remarkably, three out of 14 cats showing clinical signs most likely died as a consequence of high infection burdens of *A. abstrusus* (358,925 LPG) in combination with young age (7 months). This confirms that cats with high LPG have a higher probability of developing severe symptoms (Genchi et al., 2014). Although the actual causes leading to the death of four animals were not assessed here, it is acknowledged that young, debilitated and immunosuppressed animals may succumb to feline troglodystrogylosis (Brianti et al., 2014a; Giannelli et al., 2014a), or aeturostrogylosis (Elsheikha et al., 2016), especially when it evolves into a hyper-infection syndrome (Philbey et al., 2014), or to irreversible pulmonary hypertension (Crisi et al., 2015). For this reason, a correct diagnosis followed by an efficacious therapeutic protocol is pivotal in the management of feline lungworm infections. In this study, a single administration of the combination of fipronil, (S)-methoprene, eprinomectin and praziquantel (Broadline®, Merial) proved to be effective for the reduction of lungworm burdens and resolution of clinical signs, as already reported under experimental conditions (Knaus et al., 2014b, 2015) and in cats naturally exposed to these parasites (Rehbein et al., 2014; Giannelli et al., 2015a). Indeed, the overall efficacy of 99% against *A. abstrusus* and 100% against *T. brevior*, *O. rostratus* and *E. aerophilus* indicates that this treatment is effective for the management of these parasitic infections.

Although this study was designed to screen a large population of animals using standardized laboratory procedures, the collection of a single faecal sample from each animal might have led to an underestimation of the overall prevalence of infections, potentially surmountable by using a serological test (Briggs et al., 2013; Zottler et al., 2017). Therefore, additional studies are needed to investigate the occurrence of respiratory nematodes in other countries, to assess the overall prevalence of feline lungworms, and to further understand the epidemiological patterns of these parasites. Indeed, feline lungworms represent a topic of increasing interest in the field of veterinary sciences (Giannelli et al., 2016b), as they are widespread and sometimes life-threatening parasites of domestic and wild felids. This study contributes to a better understanding of their epidemiology. Further areas of investigations may include (i) the development of ecological and climatic models, which will allow evaluation of the impact of environmental/climatic factors on the distribution of these parasites; (ii) surveys to assess the occurrence of lungworms in their gastropod and paratenic hosts; (iii) the development of reliable morphological keys and serological diagnostic devices; (iv) the provision of information for veterinarians and owners alike on the importance of administering correct treatments and subjecting the animals to regular follow-ups.

Acknowledgements

The authors would like to thank Maria Luisa Vitale (University of Bari, Italy), Eva-Maria Zottler (University of Zurich, Switzerland) and André Pereira (Universidade Nova de Lisboa) for their support with the laboratory procedures. This work was funded by Merial SAS (Europe). FB LH and MK are currently employees at Merial. The funders had no role in data collection, data analysis, or in the preparation of the manuscript.

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