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The European wildcats (*Felis silvestris silvestris*) as reservoir hosts of *Troglostrongylus brevior* (Strongylida: Crenosomatidae) lungworms

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ABSTRACT

The increasing reports of *Troglostrongylus brevior* lungworm in domestic cats from Italy and Spain raised questions on its factual distribution and on the role wildcats play as reservoirs of these parasites. Carcasses of 21 wildcats were collected in natural parks of southern Italy (i.e., Catania, Sicily $n = 5$ and Matera, Basilicata $n = 16$) and biometrically and genetically identified as *Felis silvestris silvestris*, but two as hybrids. *Troglostrongylus brevior* and *Eucoleus aerophilus* lungworms were found in 15 (71.4%) and 7 (33.3%) individuals, respectively, being five (23.8%) co-infected by the two species. Both lungworms showed an aggregated distribution in the host population, assessed by k-index (i.e., 0.69 for *T. brevior* and 0.42 for *E. aerophilus*). Although no statistical significant difference was recorded among age, gender and geographical location of wildcats, a larger rate of infection by *T. brevior* was assessed in yearlings (85.7%) than adults (64.3%). This is the first epidemiological study reporting *T. brevior* infection in the European wildcat and discusses the potential threat this may represent for the conservation of this endangered species of felids. In addition, given the large frequency of lungworm infection herein recorded, the role of wildcats as reservoir hosts of these parasites to domestic cats is discussed.

1. Introduction

Feline lungworms represent a diverse group of nematodes, which infect the respiratory system of domestic and wildfelids. They include members of the superfamily Metastrongyloidea, which are characterized by an indirect life cycle, involving intermediate (i.e., snails or slugs) and paratenic (i.e., amphibians, birds, reptiles and rodents) hosts (Anderson, 2000). While *Aelurostrongylus abstrusus* Railliet 1898 (Strongylida: Angiostrongylidae) is regarded as the main lungworm species affecting domestic cats, other metastrongyloids such as *Troglostrongylus* spp. Vevers 1923 (Strongylida: Crenosomatidae) and *Oslerus rostratus* (Gerichter, 1949) (Strongylida: Filaridae) have been for long time regarded as parasites of wild felids (Anderson, 2000; Bowman et al., 2002). However, *Troglostrongylus brevior* (Gerichter, 1949) and *Troglostrongylus subcrenatus* Railliet and Henry 1913 have

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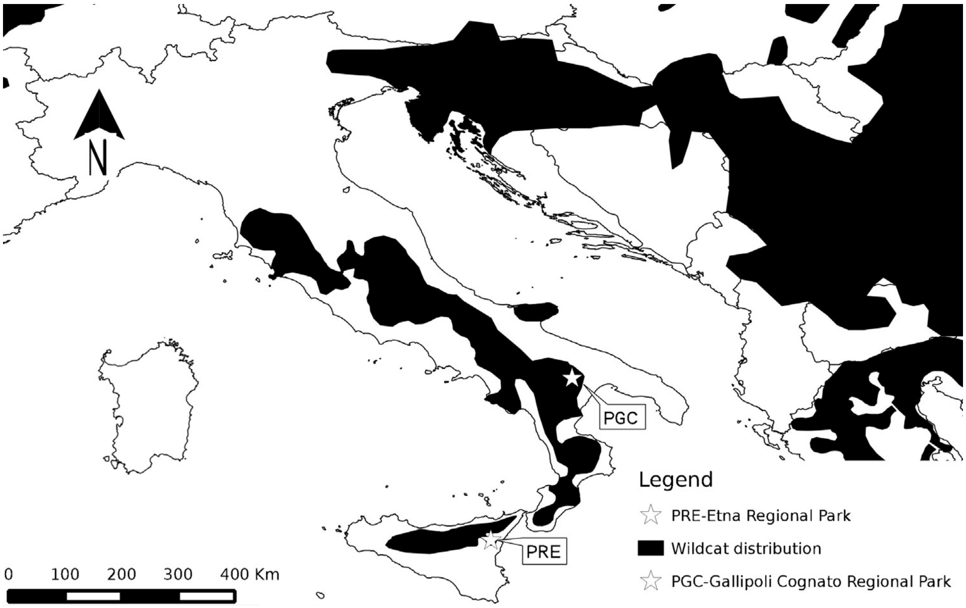

Fig. 1. Map showing the location of the two study areas, along with the wildcat distribution in Italy. *Map legend: PGC = Parco Regionale di Gallipoli Cognato; PRE = Parco Regionale dell'Etna.*

increasingly been identified in domestic cats, thereby raising hypotheses on their origin and factual distribution in feline populations (Jefferies et al., 2010b; Brianti et al., 2012, 2013; Di Cesare et al., 2013; Annoscia et al., 2014). Climate changes may affect the spread of mollusc-transmitted nematodes (Patz et al., 2000), and wild animals (i.e., fox, jackals and coyotes) have been incriminated as bridging hosts for emerging respiratory nematodes of dogs, such as *Angiostrongylus vasorum* Baillet 1866 (Strongylida: Angiostrongylidae) and *Eucoleus aerophilus* Creplin 1839 (Trichurida: Trichinellidae) (Morgan et al., 2008; Magi et al., 2009; Jefferies et al., 2010a; Takács et al., 2013). The domestic cat (*Felis silvestris catus*) and the European wildcat (*Felis silvestris silvestris*) are species taxonomically related, both belonging to the single polytypic species *Felis silvestris* Schreber 1777 (Mattucci et al., 2013). Nowadays, the European wildcat is endangered, with scattered populations living throughout Europe, including central and southern countries (Stahl and Artois, 1991). In Italy, this wild felid is mainly settled along the Apennines and in Sicily (Fig. 1), where it lives in sympatry with domestic cats (Randi et al., 2001; Mattucci et al., 2013). This overlapping distribution of both species results in a potential threat for the wildcat conservation since they may interbreed (Randi et al., 2001), produce fertile offspring, and share disease-causing pathogens (Ragni, 1993; Krone et al., 2008; Millán and Rodríguez, 2009).

In spite of the increasing number of reports of troglostrongylosis in domestic cats in Europe (Jefferies et al., 2010b; Brianti et al., 2012; 2013; Di Cesare et al., 2013; Tamponi et al., 2014), no data are available for wildcats. Indeed, information on parasites of this felid is scarce and limited to a few reports (Baruš, 1961; Hasslinger and Bortenlänger, 1996; Krone et al., 2008). This study aims to investigate the occurrence of lungworms infecting wildcats, in order to assess their impact on the health of this endangered species and to elucidate the role of wildcats as potential bridging hosts of parasites to domestic animals.

2. Material and methods

2.1. Animals and lungworm collection

From January 2005 to January 2012, carcasses of wildcats were collected in two protected natural areas of southern Italy, i.e. Parco di Gallipoli Cognato (E40.492112°, N16.158936°, Basilicata region) and Parco dell'Etna (E37.767757°, N14.975045°, Sicily) (Fig. 1). Animals killed by car accident, poacher activity, or starvation were provided for necropsy by authorized agents responsible for wildlife conservation in the Parks. Once received, carcasses were identified and frozen at −20 °C, until processed. At necropsy, animals were sexed and their age was estimated through teeth examination (Condé and Schauenberg, 1978). Biometric data, such as the body length (measured from the nasal tip to the last sacral vertebra), and weight, were recorded for each animal, as well as cat condition index (CCI), which was calculated as the ratio between weight (kg) and length (cm) (Delahay et al., 1998). A sample of the *musculus tibialis cranialis* of each wildcat was collected and molecularly processed to assess the genetic identity of animals (i.e., *F. silvestris silvestris*, *F. silvestris catus*, or hybrids) as described elsewhere (Mattucci et al., 2013). The trachea and lungs were extracted using routine necropsy techniques and examined for the presence of parasites. Briefly, the trachea and bronchi were longitudinally opened using scissors, inspected for parasites under a dissection microscope, and, thereafter, flushed with a saline solution. Lungs were inspected on their surfaces for parasite nodules, then cut in small pieces (about 1 cm^3) and squeezed in saline solution. The washing solution was collected into conic glass containers according to their anatomical portions (i.e., trachea and bronchi or lungs), decanted, rinsed until clear and observed under dissection microscope for the presence of parasites. Collected worms were washed in saline solution, fixed in 70% ethanol, mounted in glycerol and identified at species level using morphological keys (Gerichter, 1949; Anderson, 2000; Brianti et al., 2014).

2.2. Data analysis

Epidemiological indices to quantify parasite species in host populations (i.e., prevalence, mean intensity, mean abundance and the index of dispersion k) were calculated according to Bush et al. (1997). Briefly, mean intensity was calculated as the arithmetic mean of the number of parasites of a particular species per infected cats in the sample, while mean abundance as the arithmetic mean of the number of parasites of a particular species per examined cats in the sample. The index of dispersion (k) was calculated as the variance-to-mean ratio of parasite abundance. All indices were computed using Quantitative Parasitology 3.0 (Rozsa et al., 2000). Hybrids were also included in the analysis, as their behaviour is not different from that of wildcats (Germain et al., 2008). For comparison and statistical analyses, wildcats were grouped according to the area of sampling (i.e., Sicily and Basilicata), sex, and age (i.e., from 1 to 12 months, yearlings; and above 12 months, adults). Epidemiological indices were compared among population for each group to assess any significant difference. Prevalence among groups was compared using the chi-square test (Yates correction), whereas differences in mean abundance or mean intensity were tested by Whitney *U*-test. The normality distribution of data was tested by Kolmogorov–Smirnov method. Pearson correlation test was conducted between the intensity of the most abundant lungworm species and the CCI. Statistica 8.0 (Statsoft Inc., USA) was used to conduct all statistical tests and values of $p < 0.05$ were considered significant. All results are reported as mean ± standard deviation.

3. Results

A total of 21 carcasses of wildcats (12 females and 9 males) were examined ($n = 16$ from Basilicata and $n = 5$ from Sicily), being 7 yearlings and 14 adults. Based on both biometrical (Fig. 2) and molecular bases (Table 1), all wildcats were identified as *F. silvestris silvestris*, but two were identified as hybrids. The causes of mortality were road accident ($n = 14$; 66.6%), shooting by poachers ($n = 4$; 19.1%), and starvation ($n = 3$; 14.3%) (Table 1).

Troglostrongylus brevior lugworms were found in the bronchi of 15 (71.4%) wildcats and a total of 99 adult worms were retrieved in infected animals (i.e., mean abundance and mean intensity 4.7 ± 5.6 and 6.6 ± 5.7, respectively). Adults ($n = 17$) of *E. aerophilus* were recovered from the trachea and bronchi of 7 (33.3%) wildcats (i.e., mean abundance and mean intensity of 0.8 ± 1.2 and 2.4 ± 0.8, respectively). Co-infection by both species of lungworms was observed in five individuals (23.8%). An aggregated distribution in the host population was recorded for both parasites (i.e., k-index 0.69 and 0.42 for *T. brevior* and *E. aerophilus*, respectively). Epidemiological indices of both lungworm species ranked according to geographical area, sex and age of wildcats are shown in Table 2. The prevalence of infection by *T. brevior* was larger in yearlings (85.7%) than in adults (64.3%). In particular, mature worms were collected from two 1-month-old kittens (Fig. 3). Conversely, infection by *E. aerophilus* was more frequent in adults (42.9%) than yearlings (14.3%). However, for both lungworm species no statistically significant differences were recorded among epidemiological indices of groups. Intensity of *T. brevior* was negatively correlated with CCI ($R^2 = 0.0792$; $p = 0.3093$).

4. Discussion

The high prevalence of *T. brevior* infection in wildcats (71.4%) represents the largest lungworm infection rate ever recorded, either in domestic or wild felids. This finding suggests the role of wildcats as natural reservoirs for this parasite. Interestingly, *A. abstrusus* has not been found in the animal population examined, possibly indicating that wildcats are not proper hosts of this parasite. This lack of infection is somewhat surprising because both species of metastrongyloids develop in the same gastropods (Giannelli et al., 2014) and may co-infect domestic cats (Jefferies et al., 2010b; Di Cesare et al., 2013; Annoscia et al., 2014) that, in turn, should be regarded as a permissive definitive hosts.

Since its first description in *Felis ocreata* from Palestine (Gerichter, 1949), *T. brevior* had never been reported so far in wild felids from Europe, but only in domestic cats from Italy (Brianti et al., 2012; 2013; Di Cesare et al., 2013; Annoscia et al., 2014; Tamponi et al., 2014) and Spain (Jefferies et al., 2010b). The above reports open several questions on the occurrence of this parasitosis in populations of domestic cats (Otranto et al., 2013), since the similarities between *T. brevior* and *A. abstrusus* first-stage larvae might have contributed to their misidentification (Brianti et al., 2012; Otranto et al., 2013). Environmental drivers (e.g., climate-changes) have been accounted as potential causes for nurturing the spread of mollusc-transmitted nematodes in Europe (Patz et al., 2000), and wild animals, such as foxes, coyotes and jackals, have been suspected to act as bridging hosts of cardio-pulmonary nematodes (i.e., *A. vasorum* and *E. aerophilus*) to pets (Morgan et al., 2008; Jefferies et al., 2010a; Takács et al., 2013). Accordingly, based on the findings of this study and on the cases previously reported in domestic cats (Jefferies et al., 2010b; Brianti et al., 2012; 2013; Di Cesare et al., 2013; Annoscia et al., 2014), the European wildcats could be regarded as disseminators of *T. brevior* in the study areas.

No significant difference in the frequency of *T. brevior* infection was observed between the two investigated areas, indicating that the infection is widespread among populations of southern Italy where appropriate ecological niches occur. The increasing urbanization of wild areas is affecting ecological systems, therefore forcing wildcats and domestic cats to occupy the same habitats (Randi et al., 2001). This could also have unpredictable effects on the conservation of the pure wildcat populations in Europe (Suminski, 1962; French et al., 1988; Randi et al., 2001),

Fig. 2. Morphology and coat pattern in dorsal view of a pure *Felis silvestris silvestris* (left) and a hybrid *Felis silvestris silvestris* x *Felis silvestris catus* (right).

Table 1
Wildcats collected in the study. Pure = *Felis silvestris silvestris*; hybrid = *Felis silvestris silvestris* × *Felis silvestris catus*.

ID*	Region of sampling	Genetic identity	Sex	Age (month)	Weight (g)	Length (cm)	Cause of death
G01	Sicily	Pure	M	10	3200	57.0	Road killed
G02	Sicily	Pure	F	30	4480	53.0	Hunting
G03	Sicily	Pure	F	30	3100	53.0	Road killed
G04	Sicily	Pure	F	30	3100	50.0	Hunting
G05	Sicily	Pure	F	30	2800	49.5	Road killed
G06	Basilicata	Pure	M	30	2250	52.5	Road killed
G07	Basilicata	Pure	M	30	2800	57.0	Road killed
G08	Basilicata	Hybrid	M	30	3550	55.5	Hunting
G09	Basilicata	Pure	M	30	3680	55.5	Road killed
G10	Basilicata	Pure	F	18	2300	45.0	Road killed
G11	Basilicata	Pure	M	30	2850	54.0	Road killed
G12	Basilicata	Hybrid	M	30	3500	54.5	Road killed
G13	Basilicata	Pure	M	30	3400	50.0	Road killed
G14	Basilicata	Pure	M	12	2990	50.5	Road killed
G15	Basilicata	Pure	M	1	276	21.0	Starvation
G16	Basilicata	Pure	F	1	192	19.5	Starvation
G18	Basilicata	Pure	F	1	250	21.0	Starvation
G19	Sicily	Pure	M	18	1600	46.0	Road killed
G20	Basilicata	Pure	F	30	3200	51.0	Road killed
G21	Basilicata	Pure**	F	12	1600	47.0	Hunting
G22	Basilicata	Pure**	F	12	1550	46.0	Road killed

* ID are not progressive numbers since they reflect registration codes.
** Speciation of these cases was assessed only by biometrical keys.

Table 2
Epidemiological indices of lungworms species calculated in a sample of 21 wildcats grouped by geographical location, age and sex. For both lungworm species no significant differences were recorded between values of each group ($p > 0.05$).

Species	Area/group	Sample size (n)	Number infected (n)	Prevalence (%)	Mean abundance (±St. dev)	Mean intensity (±St. dev)	Intensity range (min–max)
Troglostrongylus brevior	Basilicata	16	12	75.0	4.9 ± 5.5	6.6 ± 5.4	1–19
	Sicily	5	3	60.0	4.0 ± 5.3	6.7 ± 5.5	3–13
	Yearlings	7	6	85.7	8.1 ± 4.7	9.5 ± 3.3	5–13
	Adults	14	9	64.3	3. ± 4.9	4.7 ± 6.6	1–19
	Male	10	7	70.0	5.4 ± 6.6	7.7 ± 6.8	1–19
	Female	11	8	72.7	4.1 ± 3.7	5.6 ± 1.0	3–11
	Overall	21	15	71.4	4.7 ± 5.6	6.6 ± 5.7	1–19
Eucoleus aerophilus	Basilicata	16	6	37.5	1.0 ± 1.4	2.7 ± 0.5	2–3
	Sicily	5	1	20.0	0.2 ± 0.4	1.0 ± 0.0	1
	Yearlings	7	1	11.1	0.3 ± 1.1	3.0 ± 0.0	3
	Adults	14	6	42.8	1.2 ± 1.3	2.3 ± 0.8	1–3
	Male	10	3	30.0	0.9 ± 1.4	3.0 ± 0.0	3
	Female	11	4	36.4	0.7 ± 1.1	2.0 ± 0.8	1–3
	Overall	21	7	33.3	0.8 ± 1.2	2.4 ± 0.8	1–3

and might result in an exchange of pathogens between domestic cats and wildcats, and vice versa (Krone et al., 2008; Millán and Rodríguez, 2009), as also indicated by the reports of mixed infections by *T. brevior* and *A. abstrusus* in domestic cats (Jefferies et al., 2010b; Di Cesare et al., 2013; Annoscia et al., 2014).

Although not statistically significant, the prevalence of *T. brevior* infection was larger in yearlings (85.7%) than in adults (64.3%), suggesting that young individuals are fundamental for maintaining this infection in animal populations. Noteworthy was the finding of mature worms in the bronchi of two kittens of about 4 weeks, found starved and dehydrated (i.e., not weaned) because of their mother's death. Accordingly, this finding supports the potential occurrence of vertical transmission from the infected queen to kittens (Brianti et al., 2013). Data here presented confirm the host suitability of the European wildcat for *E. aerophilus* (Krone et al., 2008), and also show the largest prevalence ever recorded for this parasite (i.e., 33.3%). Differently from *T. brevior*, the retrieval of the largest frequency of infection in adults may also reflect a different transmission pattern of *E. aerophilus*. Indeed, *E. aerophilus* is transmitted throughout the ingestion of environmental larvated eggs, or via heartworms which act as facultative transporting hosts (Anderson, 2000). Therefore, the likelihood of being infected increases proportionally with animal age, having adult animals more chances for acquiring the infection.

This study represents the first large survey on lungworms of *F. silvestris silvestris*, which is an endangered animal species. It is worth noting that the majority of collected wildcats died for human related actions (i.e., 66.6% road killed, 19.1% shot by poachers), as already reported in central Europe, where road casualties (80%) and illegal hunting (6.7%) represented the main causes of death in 15 European wildcats (Krone et al., 2008). In spite of the large prevalence for lungworms herein recorded, an aggregated distribution pattern (Wilson et al., 2002) for both species was assessed. Since the parasite richness is considered an indicator of wild animals' fitness and biodiversity (Lafferty, 2012), results of the present study suggest that wildcat populations are not threatened by lungworm infections. On the other hand, the negative correlation observed between *T. brevior* intensity and CCI ($R^2 = 0.0792$, $p = 0.3093$) indicates

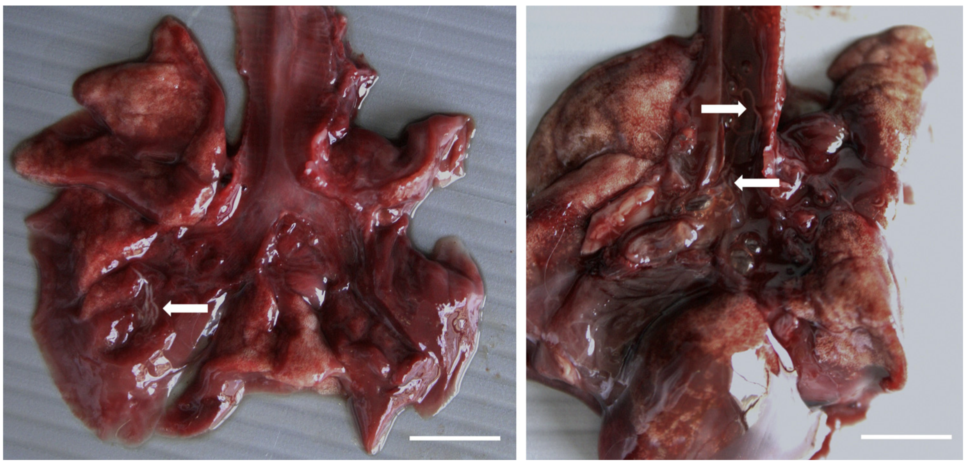

Fig. 3. Adult worms of *Troglostrongylus brevior* (arrows) in the lungs of two European wildcat (*Felis silvestris silvestris*) kittens of four weeks of age. Scale bar = 1 cm.

that the higher the number of *T. brevior* in the lungs, the lower the condition index. Nonetheless, other concomitant conditions impacting on the CCI (e.g., intestinal parasites, viral or bacterial infectious diseases) cannot be ruled out, and further investigations are required to elucidate the role of lungworms in determining impaired health conditions.

In conclusion, results of the present survey indicate that wildcats may be highly infected with *T. brevior* and *E. aerophilus* and might act as reservoirs of these parasites to sympatric domestic animals. In addition, data herein reported support the hypothesis of a direct transmission of *T. brevior* from queen to non-weaned kittens, thus shedding lights on the ecology of this neglected metastrongyloid. Based on the topography, climatic features and prey spectrum of geographical regions where wildcats were collected, a similar picture might be envisaged in wildcat populations living across the Mediterranean basin. Further surveys on animals from different geographical areas are required to elucidate the epidemiology of troglostrongylosis in European wildcats as well as in the domestic cats.

Conflict of interest statement

Authors declare to not have any financial and personal relationships with other people or organisations that could inappropriately bias the present study.

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