



# Serological survey and risk factors of *Aelurostrongylus abstrusus* infection among owned cats in Italy

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## Abstract

Feline lungworms affect the respiratory tract of domestic cats causing respiratory conditions of various degrees. In this study, we investigated the exposure of cats to feline lungworm infections by detecting antibodies in a large population of animals from several regions of Italy. Sera of 1087 domestic cats living in regions of the north ( $n = 700$ ), the centre ( $n = 227$ ) and the south ( $n = 160$ ) of Italy were examined by a newly developed indirect ELISA conceived for detection of antibodies against the most frequently occurring feline lungworm *Aelurostrongylus abstrusus*. Individual cat data (i.e., age, sex, neutering status and provenience) were analysed as potential risk factors for exposure to lungworm infections. Samples were additionally screened for feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) proviral DNAs. Overall, 9% (98/1087; 95% confidence interval (CI) 7.4–10.9%) of the animals tested seropositive to lungworm antibodies. Positive cats were identified in the north (7.1%; CI 5.5–9.3%), in the centre (5.3%; CI 3.0–9.0%) and in the South (22.5%; CI 16.7–29.6%), with more seropositive animals in the latter area ( $p < 0.05$ ). The risk of lungworm infection in cats was significantly associated with age less than 6 months (i.e. 24.4%,  $p < 0.05$ ) and FIV infection ( $p < 0.05$ ). This large-scale serological survey confirms the exposure of cats to lungworm infections in Italy and that serological tests can be used to assess the distribution of lungworm infections in large populations of animals.

**Keywords** Lungworms · Italy · Cat · ELISA · Antibodies · Prevalence · *Aelurostrongylus abstrusus*

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## Background

Wild and domestic felids may be parasitized by metastrongyloid lungworms, which cause respiratory conditions of various degrees (Beugnet et al. 2014; Veronesi et al. 2016; Giannelli et al. 2017). Besides *Aelurostrongylus abstrusus* (Metastrongyloidea, Angiostrongylidae), which is the most frequently occurring feline lungworm, other species such as *Troglostrongylus brevior* (Metastrongyloidea, Crenosomatidae) and *Angiostrongylus chabaudi* (Metastrongyloidea, Angiostrongylidae) have recently extended the list of gastropod-borne nematodes affecting domestic cats in single areas (Brianti et al. 2013; Varcasia et al. 2014; Colella et al. 2017). After being firstly described in wild felids in Palestine (Gerichter 1949) and, then, in cats in 2010 (Jefferies et al. 2010), *T. brevior* has been increasingly detected in domestic cats with a prevalence up to 10.8% in Bulgaria (Giannelli et al. 2017). Another lungworm species, namely *Oslerus rostratus* (Metastrongyloidea, Filaroididae), has been scantily reported through case report studies (Juste et al. 1992; Brianti et al. 2014; Varcasia et al. 2014) and with low prevalence (i.e. 0.4%) in a comprehensive investigation performed in Europe (Giannelli

et al. 2017). These metastrongyloids are characterized by an indirect life cycle involving terrestrial mollusks as intermediate hosts, in which first-stage larvae (L1), released in the faeces of infected hosts, develop to the infective third-stage larvae (L3) (Hobmaier and Hobmaier 1935; Giannelli et al. 2014). Infection of cats occurs through the ingestion of gastropods (e.g. *Cornu aspersum*; Giannelli et al. 2014) or, more likely, vertebrate and invertebrate species acting as paratenic hosts (e.g. mice and cockroaches; Falsone et al. 2017; Colella et al. 2019). Alternative transmission pathways include the release of L3 in the mucus and/or from dead snails submerged in water (Giannelli et al. 2015) and snail-to-snail transmission, named as *intermediensis* (Colella et al. 2015). Additionally, vertical transmission of *T. brevior* from the queen to the litter has been hypothesised (Brianti et al. 2013). These transmission pathways contribute to the increase of exposure to lungworm infections of both wild and domestic cats. Once infected by *A. abstrusus* and/or *T. brevior*, cats may develop a low respiratory tract disease characterized by a wide range of respiratory signs including chronic cough, sneezing and nasal discharge (Brianti et al. 2014; Elsheikha et al. 2016; Cavalera et al. 2018; Schnyder et al. 2014) but also unspecific clinical signs such as anorexia, lethargia, weight loss, enlarged lymph nodes or diarrhoea (Crisi et al. 2017; Genchi et al. 2014; Schnyder et al. 2014). Severe dyspnoea can lead to the exitus, especially in young animals (Cavalera et al. 2018; Genchi et al. 2014; Crisi et al. 2017). Instead, limited data available on clinical features of *O. rostratus* and *A. chabaudi* still impair knowledge on the outcome upon infection with these parasites in wild and domestic cats (Varcasia et al. 2014; Giannelli et al. 2017).

The diagnosis of lungworm infections in cats is mainly achieved by the detection of L1 in the faecal samples using a larval migration method such as the Baermann-Wetzel technique. Though this is the most employed test for the diagnosis of metastrongyloid infections (Giannelli et al. 2017), it has several limitations such as the relatively long time of execution (up to 24 h), the need for fresh and individual faecal samples (Abbate et al. 2018; Zottler et al. 2017), the need of operator skills in identifying the species of L1, the absence of L1 during the prepatent period and the time-limited and intermittent larval shedding especially in chronic infections (Hamilton and McCaw 1968; Schnyder et al. 2014; Cavalera et al. 2019). Therefore, a recently developed method detecting anti-*A. abstrusus* antibodies in serum samples based on an enzyme-linked immunosorbent assay (ELISA) has been standardized (Zottler et al. 2017) allowing mass screenings of cat serum samples for epidemiological surveys (Gueldner et al. 2019; Alho et al. 2018). This serological test employs the recombinant major sperm protein (MSP) of the cattle lungworm *Dictyocaulus viviparus* (Trichostrongyloidea, Dictyocaulidae). However, though this ELISA has been developed for the detection of antibodies against *A. abstrusus*, cross-reactions with other feline lungworms such as *T. brevior* or *O. rostratus* cannot be ruled out (Zottler et al. 2017).

Considering the lack of information about the seroprevalence of feline lungworms, this study represents the first serological survey to assess the exposure to lungworms in a large population of cats across northern, centre and southern regions of Italy.

## Materials and methods

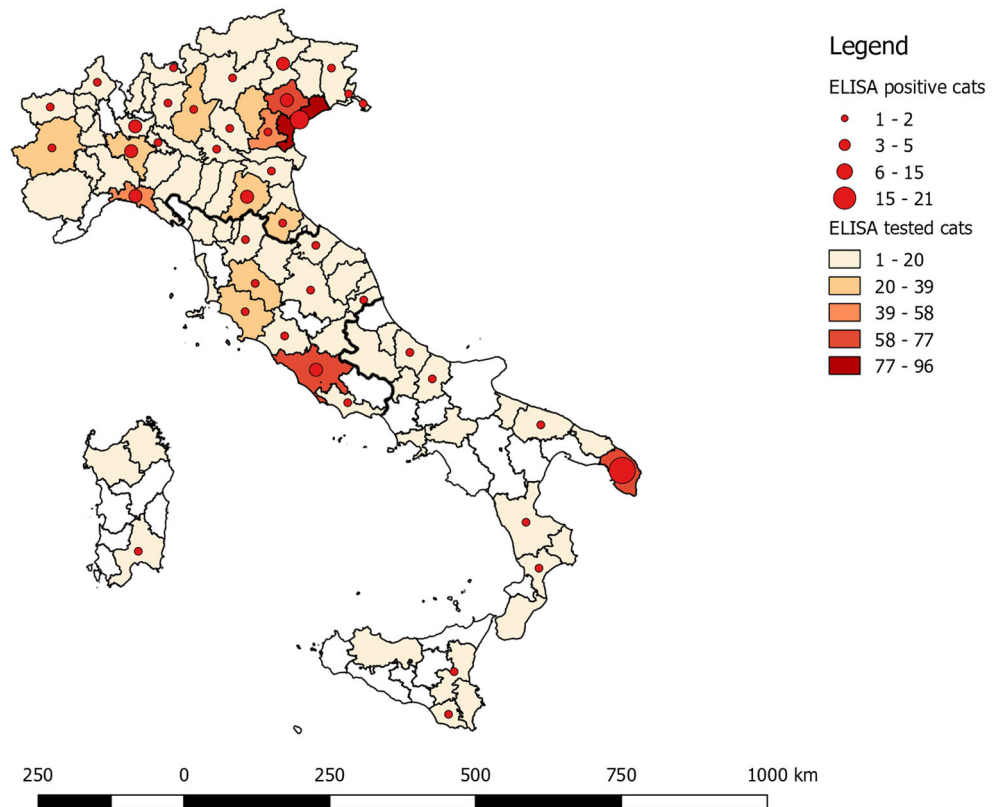
From June to December 2017, serum samples were obtained from 1087 owned cats living in the north ( $n = 700$ ), the centre ( $n = 227$ ) and the south ( $n = 160$ ) of Italy, which were referred to laboratories for veterinary analyses (Fig. 1). Individual cat data (i.e., age, sex, neutering status and provenience) were recorded. Animals were grouped according to their age as follows: (i)  $\leq 6$  months (paediatric age), (ii)  $> 6 < 24$  months and (iii)  $> 24$  months. No other information was available about the occurrence of intestinal or extra-intestinal parasites in the population of cats. Sera were analysed at the Institute of Parasitology of the University of Zurich, Switzerland, for antibodies against lungworms by ELISA (sensitivity 82%, specificity 90%; Zottler et al. 2017) as previously described (Gueldner et al. 2019). Test thresholds were determined with 300 randomly selected samples based on the mean value of optical density (OD) plus three standard deviations, and a reference serum was added twice on each plate to calculate a correction factor for adjustment between plates (Schnyder et al. 2011). Samples ( $n = 993$ ) were also screened for feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) proviral DNAs as previously described (Endo et al. 1997; Stiles et al. 1999) at the Department of Veterinary Medicine, University of Bari. The protocol of this study was approved by the Ethical Committee of the Department of Veterinary Medicine of the University of Bari (Prot. no. 7/17).

The locations of all serum samples and of those that scored ELISA-positive were geo-referenced and mapped, according to owner's province, using an open-source geographical information system (Quantum GIS 2.2, [www.qgis.org](http://www.qgis.org)). Excel for Windows (Microsoft Corporation, Redmond, USA) was used to calculate the prevalence values and their 95% confidence intervals (CI). The association between seroprevalence and provenience, age, sex, neutering status and FIV or FeLV infection was investigated by a univariate statistical analysis using Pearson's chi-square test ( $\chi^2$ ). A value of  $p < 0.05$  was considered statistically significant. The statistical analyses were performed using GraphPad Prism version 8.0.0 (GraphPad Software, San Diego California, USA).

## Results

Out of 1087 analysed cat sera, 98 (9%; CI 7.4–10.9%) were seropositive. The number of positive animals according to provenience, age classes, sex and neutering status is shown in Table 1. Antibodies were found in 45.2% (42/93) of the

**Fig. 1** Geographical representation of provinces from where serum samples were collected (coloured polygons) and number of ELISA-positive cats according to provinces (graded red dots)



investigated provinces confirming that the infection spread throughout the country (Fig. 1) with different proportions in northern (7.1%; CI 5.5–9.3%), centre (5.3%; CI 3.0–9.0%) and southern (22.5%; CI 16.7–29.6%) regions. The number of positive cats was significantly higher in the south (i.e. 22.5%,  $p < 0.05$ ) than in the other parts of Italy (Fig. 1) and in animals of paediatric age (i.e. 24.4%,  $p < 0.05$ ). Based on univariate analysis, no further statistically significant differences were detected between sex of animals or their neutering status and the ELISA positivity. Moreover, 52 and 26 cats were positive for FeLV (5.2%) and FIV (2.6%), respectively, of which 9.6% (5/52) for FeLV and 26.9% (7/26) for FIV were also lungworm seropositive. Lungworm seropositivity was significantly associated with FIV infection ( $p < 0.05$ , Table 1). Out of 5 cats co-infected with FeLV and FIV, three were also positive for antibodies against lungworms.

## Discussion

This study represents the first large serological survey to assess the nationwide exposure of cats to feline lungworm infections in Italy. The herein adopted ELISA was conceived for the detection of antibodies against *A. abstrusus*, which is the most frequently occurring metastrongyloid lungworm throughout Europe, including Italy (Giannelli et al. 2017). However, cross-reactions with other feline lungworms such as *T. brevior* or *O. rostratus*

cannot be ruled out (Zottler et al. 2017), particularly in areas (e.g. Italy) where these lungworms co-exist (Giannelli et al. 2017; Di Cesare et al. 2015). Therefore, further studies including experimentally infected cats are needed to understand seropositivity to other metastrongyloids. However, potential cross-reactions with *O. rostratus* are of less concern considering that this parasite is rarely diagnosed in domestic cat populations (Giannelli et al. 2017). Similarly, cross-reactions against the trichurid and, therefore, taxonomically not closely related respiratory nematode *Capillaria aerophila* (syn. *Eucoleus aerophilus*) are unlikely (Zottler et al. 2017). A 9% seroprevalence indicates that these cats were having an active lungworm infection and/or had previous contact with metastrongyloids, since seroconversion occurs at the earliest 15 days post-infection and may persist until 4–8 weeks after anthelmintic treatment (Zottler et al. 2017). ELISA-positive cats were found all over the country confirming the endemic occurrence of lungworms in Italy (Giannelli et al. 2017). However, the number of positive animals was significantly higher in the south (i.e. 22.5%,  $p < 0.05$ ) than in the other parts of Italy suggesting that cats in the southern regions are more exposed to feline lungworms. Similarly, previous studies on populations of cats in Italy based on coprological analyses showed a higher prevalence of lungworm infection in southern (i.e. 14.5%; Giannelli et al. 2017) than in northern (i.e. 8.6%; Di Cesare et al. 2015) regions. These results could be related to climatic and ecological factors (e.g. temperature and humidity) in selected suitable endemic areas affecting the development of the

**Table 1** Risk factor analysis for lungworm infection in cats from Italy based on serological antibody detection

	Seropositive cats/no. of cats	Prevalence (%) (95% CI)	Chi-square test <i>p</i> value
Provenience			
North	50/700	7.1 (5.5–9.3)	<b>&lt; 0.0001</b>
Centre	12/227	5.3 (3.0–9.0)	
South*	36/160	22.5 (16.7–29.6)	
Sex			
Females	45/552	8.1 (6.1–10.7)	0.312
Males	53/535	9.9 (7.6–12.7)	
Age			
< 6 months*	11/45	24.4 (14.2–38.7)	<b>&lt; 0.0001</b>
6–24 months	23/199	11.5 (7.8–16.7)	
> 24 months	64/843	7.6 (5.9–9.6)	
Neutering status			
Intact	7/91	7.7 (3.8–15.0)	0.769
Neutered	78/908	8.6 (6.9–10.6)	
No info <sup>a</sup>	13/88	14.8 (8.8–23.6)	
Other infections			
FeLV+	5/52	9.6 (4.2–20.6)	0.736
FeLV–	78/941	8.3 (6.7–10.2)	
FIV+*	7/26	26.9 (13.7–46.1)	<b>&lt; 0.0001</b>
FIV–	76/967	7.9 (6.3–9.7)	

Significant *p* values are printed in bold

\*Significant compared with the other variables

<sup>a</sup> Not considered for statistical analysis

lungworms inside their intermediate hosts (Yousif and Lämmler 1975; Ferdushy et al. 2010). Additionally, the selection of the study population and the sample size may also bias a comprehensive evaluation of metastrongyloids epidemiology.

No statistically significant differences were found in the seroprevalences of cats from northern (i.e. 7.1%) and centre (i.e. 5.3%) regions of Italy. However, the great number of cats enrolled from the north ( $n = 700$ ) might have likely influenced the general prevalence in Italy (i.e. 9%) that was comparable with the one recently recorded in Switzerland (i.e. 10.7%; Gueldner et al. 2019) using the same serological test. Interestingly, in this latter study, the subpopulation aging 11–22 months was significantly more often positive (13.2%, CI 6.5–22.9%) than younger or older animals, while here, a significant proportion of seropositive animals was found in paediatric cats up to 6 months (24.4%; CI 14.2–38.7%;  $p < 0.05$ ) compared with other age classes. Though the limited sample size of this group of animals ( $n = 45$ ), this finding may be related to an active infection by *T. brevior*, which is more prevalent in paediatric cats than other lungworm species (Cavalera et al. 2018) also as a reflection of its likely vertical transmission pathway (Brianti et al. 2013). In accordance with previous studies (Beugnet et al. 2014; Giannelli et al. 2017; Gueldner et al. 2019), no statistical association was found between the number of ELISA-positive cats and sex of

animals (Table 1). Similarly, the neutering status did not influence the probability of a cat to be exposed to lungworm infections (Table 1), though in a previous study was suggested that intact cats were at higher risk of infection than neutered ones (Gueldner et al. 2019). Considering that the neutering status has been rarely taken in consideration as a risk factor in the past (Gueldner et al. 2019), further studies are needed to better understand its role in the epidemiology of feline lungworm infection. In this study, seropositivity to lungworm was significantly associated with FIV infection ( $p < 0.05$ ). This viral immunosuppressive infection is the result of a cat-to-cat contact, for example through bite wounds, implying that outdoor access is likely. This living condition can also increase the chances for cats to acquire lungworms as well as intestinal and extra-intestinal parasites (Beugnet et al. 2014).

## Conclusions

The use of ELISA for the detection of antibodies against lungworms in a large population of cats from Italy allowed a first assessment of the exposure of cats to these infections. The application of this tool provided evidence on the occurrence of lungworms not only in areas where they are already



considered endemic (e.g. Italy) but also in areas where their prevalence is apparently low or non-investigated due to a non-routine use of the Baermann method. For treatment purposes, a definitive diagnosis may be achieved by combining the ELISA with the clinical picture and, if possible, with a direct test to confirm the presence of a current infection.

### Compliance with ethical standards

The protocol of this study was approved by the Ethical Committee of the Department of Veterinary Medicine of the University of Bari (Prot. no. 7/17).

**Conflict of interest** The authors declare that they have no conflict of interest.

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