Prevalence of feline lungworm *Aelurostrongylus abstrusus* in England

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Abstract

Infection of cats with lungworm *Aelurostrongylus abstrusus* has recently been documented in the UK. Here, we aimed to study the prevalence of *A. abstrusus* in fecal samples from cats across England. A total of 950 fecal samples were collected from cats together with information on their age, breed, gender, geographic region, lifestyle, and treatment history. A total of 17 (1.7%) cats were positive for *A. abstrusus* based on species-specific morphological features of the larvae isolated by Baermann's technique. There was no statistically significant difference in the proportion of positive samples between females (506; 53.2%) and males (444; 46.7%). Multiple regression analysis showed that prevalence of feline lungworm was significantly different across geographic regions: in comparison with East Midlands, some regions had shown significantly increased odds of *A. abstrusus*-positive samples (South East [odds ratio [OR] = 7.68; 95% confidence interval [CI] = 1.70 to 32.76; $p = 0.01$]; West Midlands [OR = 6.20; 95% CI = 1.21 to 26.84; $p = 0.02$]), while other regions had also increased odds although not statistically significant (Greater London [OR = 9.63; 95% CI = 0.43 to 84.05; $p = 0.07$]; North West [OR = 4.25; 95% CI = 0.59 to 20.89; $p = 0.09$]; South West [OR = 2.48; 95% CI = 0.12 to 17.64; $p = 0.43$]; and North East [OR = 1.88; 95% CI = 0.10 to 12.24; $p = 0.57$]). Keeping cats inside was protective against the risk of infection compared with those having outdoor access (OR = 0.09; 95% CI = 0.01 to 0.48; $p = 0.02$). On the other hand, age, breed, gender and deworming history did not have any significant effect on the likelihood of infection. Our data indicate that *A. abstrusus* is a parasite of potential significance in cats, in particular those from certain geographic regions in England. To reduce the spread of this parasite, an integrated feline lungworm control program needs to be implemented.

Keywords: Cats; lungworm; *Aelurostrongylus abstrusus*; prevalence; survey; risk factors

1. Introduction

The gastropod-borne nematode *Aelurostrongylus abstrusus* (Railliet, 1898) is the most common lungworm of domestic and wild felids, and is found in many parts of the world, including Europe, USA, South America and Australia (Scott, 1973; Elsheikha et al., 2016; Giannelli et al., 2017; Penagos-Tabares et al., 2018). This parasite has a considerable impact on the health and welfare of cats. Also, it has shown both regional endemicity and geographic expansion across Europe. Infected cats exhibit chronic wasting, cough, dyspnea, pulmonary wheezes and other signs of lower airway disease, although asymptomatic cases, shedding high number of larvae in feces, may also occur (Genchi et al., 2014; Elsheikha et al., 2016; Hansen et al., 2017). In addition to *A. abstrusus*, recent studies have detected other metastrongyloids, such as *Troglostrongylus brevior* (Crenosomatidae) and *Oslerus rostratus* (Filaroididae) and the trichurid *Eucoleus aerophilus* (syn. *Capillaria aerophila*) in the lungs of cats (Pennisi et al., 2015; Giannelli et al., 2017).

Biological and epidemiological drivers (Traversa et al., 2009; Beugnet et al., 2014; Hansen et al., 2017), some of them yet unconfirmed, appear to be increasing the risk of infection in cats in certain parts of the world. However, important gaps remain in the available literature surrounding the prevalence of feline lungworm infection and its epidemiological patterns as well as determinants. Lack of understanding of these changing patterns may have serious implications from a clinical standpoint, given that a delay in diagnosis and treatment can lead to severe lesions and even death of the infected cat. Recently, more cases have begun to be observed by clinicians (Gunn-Moore and Elsheikha, 2018). Despite this increasing frequency of *A. abstrusus* in cats, there is lack of epidemiological studies that assess the prevalence and distribution of this parasite in cats in The UK. A pan-European study involving 12 countries, reported 0% *A. abstrusus* infection rate in fecal samples collected from Cambridge, UK (Giannelli et al., 2017). However, this study is not representative to the status of *A. abstrusus* infection in The UK due to its very small sample size. Given the paucity of data on *A. abstrusus* in The UK, a larger survey involving more samples collected from diverse geographic areas is needed in order to provide important insight into the transmission potential of *A. abstrusus* in cats.

We previously conducted a cross-sectional survey in England and in the initial phase we detected *A. abstrusus* larvae in the feces of 2.2% (14 out of 629) of cats (Elsheikha et al., 2017). Herein, we report a more up-to-date *A. abstrusus* prevalence rate, after the completion of the survey, based on the analysis of 950 fecal samples from cats across seven main geographic regions of England. Our study established a new background prevalence of *A. abstrusus* in cats in England and identified outdoor access as a potential risk factor for *A. abstrusus* infection. This new knowledge may lead to more insight into the real burden and risk of feline lungworm infection in the UK, which will ultimately lead to improved sustainable management strategies for feline aelurostrongylosis.

2. Materials and methods

2.1. Fecal samples and data collection

From January 2016 to January 2018, fecal samples ($n = 950$) were collected from cats, 506 females and 444 males, across seven administrative regions of England. The study was designed to include feral and street cats in addition to domestic cats. Fecal samples were collected from cats from shelters, catteries and privately owned cats, and were examined using Baermann's technique in order to isolate the first stage larvae (L1s). Morphological identification of the isolated *A. abstrusus* L1s and its differentiation from L1s of other metastrongyloids was achieved via microscopic examination using previously described morphometric features of *A. abstrusus* larvae (Gerichter, 1949; Brianti et al., 2014; Giannelli et al., 2014; Giannelli et al., 20117).

Data on age (kitten [0 – 6 months]; junior [7 months – 2 years]; prime [3 years – 6 years]; mature [7 years – 10 years]; and senior [11 years – 14 years]), breed, gender (male vs female), main geographic regions in England (North East, North west, South East, South West, East Midlands, West Midlands, and Greater London), lifestyle (indoor, outdoor access, feral, and stray) and deworming history (recently treated using anthelmintics, such as emodepside [Profender®] or macrocyclic lactones, which have reported efficacy against A. *abstrusus* vs left un-treated) were collected. Ethical approval was granted by the Research Ethics Committee of School of Veterinary Medicine and Science, University of Nottingham.

2.2. Prevalence and risk factor analyses

Statistical relationships were assessed between fecal shedding of *A. abstrusus* larvae and defined risk factors, such as age, breed, gender, geographic location, cat lifestyle, and animal treatment status at the time of fecal sampling. The overall parasite prevalence was determined by dividing the number of parasite-positive fecal samples by the total number of samples collected within each risk factor category. Test of independence for contingency tables was used to evaluate associations between each risk factor (e.g., animal age, breed, gender etc.) and presence of *A. abstrusus* larvae. Multiple logistic regression was used to investigate the associations between host-specific, demographic, and environmental risk factors with respect to test outcome (e.g., parasite present or absent). Risk factors that were significant at a p level of < 0.1 were then incorporated in a forward-stepping manner into multiple logistic regression models. These multivariable models yielded adjusted odds ratios (OR) that simultaneously measured the strength of associations between multiple risk factors and the presence of parasite larvae in cat's feces.

2. Results and discussion

2.1. Characteristics of the cat population

A total of 950 cats were examined with ages ranging from 2 to 240 months (mean age 53.1 ± 37.7 months). Approximately, 53.2% (506) of the cats were females and 46.7% (444) were males. Breed distribution included 910 (95.78%) domestic short hair, 27 (2.84%) domestic longhair and 13 (1.36%) belonged to British Semi longhair (n = 6), British longhair (n =3), Bengal (n =1), Burmese (n =1), Cornish Rex (n =1), and Maine Coon (n =1).

2.2. Prevalence of and risk factors associated with infection

In our study, 1.7% (17/950) of the fecal samples tested were positive. According to a recent epidemiological survey conducted across 12 European countries, feline lungworms were the second most frequent group of nematodes diagnosed in cats, and *A abstrusus* was the most frequently detected lungworm species across Europe, but none of the samples tested from cats in Cambridge (UK) was positive for *A. abstrusus* (Giannelli et al., 2017). However, the 0% prevalence reported previously from Cambridge may not be representative of the cat population in The UK due to the very small sample size examined. In fact, prevalence obtained in our study seems to fall within the prevalence range reported in Europe, which varied greatly from 0.38% in Croatia (Grabarević et al., 1999), 1% in Germany (Mundhenke and Daugschies, 1999) 2.08% in Ireland (Garcia-Campos et al., 2018), 2.3% in Switzerland (Zottler et al., 2019), 2.6% in the Netherlands (Robben et al., 2004), 8.3% in the Denmark (Hansen et al., 2017), 26.5% in in Italy (Genchi et al., 2014), to 43.1% in Albania (Knaus et al., 2011).

The prevalence rate of *A. abstrusus* can also vary significantly within the same country, for example in Denmark local prevalence rates varied from 0% [95% CI: 0.0–8.8] to 31.4%

[95% CI: 16.9–49.3] (Hansen et al., 2017). Difference in prevalence rates was also detected among three regions in Italy (Giannelli et al., 2017). A similar trend was detected in our study where significant differences were observed in the geographic regions in regard to their association with the increase in the odds of *A. abstrusus* infection, in comparison with East Midlands region (Table 1). The disparity among the prevalences of feline lungworm *A. abstrusus* across geographic regions may reflect the level of transmission or availability of intermediate and paratenic reservoir hosts that are able to maintain *A. abstrusus* life cycle in certain areas. The broad geographic distribution of *A. abstrusus* in our study indicates that *A. abstrusus* is circulating in cat population and not restricted to a certain locality in England. Further epidemiological studies are required to determine the factors that drive the transmission of *A. abstrusus* in the areas where this parasite is highly prevalent.

In this study, the larvae per gram of feces (LPG) were determined using Baermann's technique and ranged from 8 to 22 (11.6 ±3.3). This was surprisingly low compared to a previous study that detected a mean of 508.7 LPG (Giannelli et al., 2017). The larval survival tend to decline, due to dehydration, depending on the cat litter type and the duration of time faecal samples remain in the litter; a reduction in the viability of 80% of larvae occurred after 3 h and reached almost 100% after 24 h (Abbate et al., 2018). In our study, the length of time samples remained in the litter before collection ranged from 1 to 4 h. Thus, we must be cognizant of the potential influence of dehydration on the larval viability, given the low-parasitic load and the time elapsed while the samples are present in the cat litter, which may have underestimated the isolation rate of larvae in our study. It is also worth mentioning that although Baermann's technique is specifically used for direct isolation of lungworm larvae from feces, its diagnostic performance and sensitivity can be compromised by various factors. These include the inability to isolate larvae in the pre-patent period, inconsistent shedding of the larvae especially in cases with low parasite burdens, or cessation of shedding larvae by some cats, despite being infected, which in turn lead to false negative results (Hamilton, 1968; Elsheikha et al., 2016). To increase the accuracy of detection of *A. abstrusus* in future surveys, Baermann's technique should be performed on freshly voided fecal samples collected on three consecutive days. Serological detection of antibodies has dramatically improved the sensitivity of detection of lungworms (Zottler et al., 2017), adding more value for the diagnosis of feline aelurostrongylosis. Thus, a greater emphasis on the use of a serological assay in conjunction with fecal analysis may be warranted to achieve more accurate laboratory diagnosis.

We examined the association between age, breed, gender, lifestyle, deworming history, and the risk of infection with *A. abstrusus*. Our risk assessment analysis did not detect any effect of the age or breed on the frequency of infection. However, the risk of *A. abstrusus* infection in Denmark was lower in kittens younger than 11 weeks compared to older cats (Hansen et al., 2017). Another study reported significantly higher prevalence in cats younger than 2 years and in cats co-infected with other gastrointestinal parasites (Giannelli et al., 2017). In the present and previous studies, gender was not a risk factor for infection with *A. abstrusus* (Traversa et al., 2008; Barutzki and Schaper, 2013; Olsen et al., 2015; Hansen et al., 2017). Also, we did not detect any differences between neutered and intact cats

Out of the 17 infected cats, 13 had outdoor access, three were stray cats and one was an indoor cat (Table 2). Keeping cats indoor was associated with significant protection against infection (odds ratio [OR] = 0.09; 95% confidence interval [CI] = 0.01 to 0.48; $p = 0.02$). In contrast, no significant difference was detected between stray cats and cats with outdoor access (OR = 1.01; 95% CI = 0.22 to 3.49; $p = 0.99$); probably due to the small number of cats in these categories. These findings lend further support to previously reported findings where rural origin, feral lifestyle and outdoor access have been shown to correlate with an increased risk of infection (Traversa et al., 2009; Beugnet et al., 2014; Hansen et al., 2017).

Out of the 17 infected cats, four cats were treated with various anthelmintics on the day of sample collection and 13 cats were non-treated. Our analysis has shown a lack of correlation between deworming history and risk of infection. Although deworming was not associated with a significant reduction in the infection risk, treatment is still a key factor that influences the frequency of infection with lungworm in cats. This is because many cats with outdoor access have more opportunity to acquire infection by preying on intermediate and transport hosts. Also, the frequency and timing of deworming are likely to affect the likelihood of infection. Additionally, these results should be interpreted with caution given the small number of positive samples in the stratified categories, which may not have been sufficient to identify any protective effect of deworming.

In conclusion, the present study addressed a significant aspect of the epidemiology of *A. abstrusus*, a potential serious health problem in feline medicine. Our findings demonstrate that *A. abstrusus* is present in 1.7% of cats in England, and infection frequency seems to vary according to the geographic region and lifestyle. These findings suggest that *A. abstrusus* should be considered a potential cause of respiratory tract disease in cats presenting with pulmonary manifestations including cats with mild respiratory signs. However, it is possible that cats can be infected and shed high number of larvae in feces without presenting clear clinical signs. Therefore, integrated strategies for management of *A. abstrusus* as well as other feline metastrongyloid lungworms (*Troglostrongylus* spp., *Oslerus rostratus*, *Capillaria aerophila*), should be implemented and can be achieved through using preventative anthelmintics, enhanced diagnostics and increased awareness of feline lungworms. To this end, awareness and education campaigns, launched by pharma and professional organizations, such as European Scientific Counsel Companion Animal Parasites (ESCCAP) and Companion Animal Parasite Council (CAPC), which promote adherence to lungworm prophylaxis should be tailored to at-risk cat populations.

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Table 1.

The prevalence of *A. abstrusus* across seven administrative regions in England.

Geographic region	Prevalence*	Odd ratios	95% CI	*p*-value
Greater London	1/17 (5.8)	9.632	0.43 to 84.05	0.07
South East	4/72 (5.5)	7.68	1.70 to 32.76	0.01
West Midlands	3/69 (4.3)	6.2	1.21 to 26.84	0.02
North West	2/57 (3.5)	4.25	0.59 to 20.89	0.09
South West	1/46 (2.1)	2.48	0.12 to 17.64	0.43
North East	1/49 (2.0)	1.88	0.10 to 12.24	0.57

* Using East Midlands as a reference [5 infected out of 623 tested (80.2%)], Greater London, North West, South East, West Midlands showed significant differences. Prevalence is shown as number of positive samples/total number tested (%).

Table 2.

Lifestyle distribution and positivity rates of *A. abstrusus* in cats examined in this study.

Correlation was established only between cats living indoor and *A. abstrusus* infection.

Lifestyle category	**No. of uninfected cats**	**No. of infected cats**	**Larvae per gram of feces**
Indoor	375	1	12.0
Outdoor Access	455	13	11.5
Stray	81	3	12.0
Feral	22	0	0.0
Total (*n* = 950)	933	17	11.6