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Investigation of the Prevalence of Lungworm According to Fecal Examination in Cats in Kırklareli Region/Türkiye

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ABSTRACT

The aim of this study is to investigate the prevalence of lungworm according to fecal examination in cats in Kırklareli region. For this purpose, stool samples were taken from the defecation areas of 100 owned and stray cats in the Kırklareli region. Stool samples taken were delivered to Kırıkkale University, Faculty of Veterinary Medicine, Routine and Epidemiology laboratory in accordance with the cold chain rules. Stool samples were examined for lungworm using the Fülleborn Flotation technique and the Baermann technique. McMaster test was used to determine the number of larvae/eggs per gram of feces, which were positive for the larvae of lungworm. As a result of the study, *Aelurostrongylus abstrusus* larvae, one of the lungworm of cats, were found in 8 (8%) of the cat feces examined by the Baermann method, while all feces were negative in the Fülleborn flotation method. There was no statistically significant difference in terms of *A. abstrusus* positivity according to age, breed and gender. A minimum of 50 and a maximum of 500 larvae were found in per gram of feces using the McMaster technique. In conclusion, this study is the first to determine lungworm in cats in Kırklareli region. It has been demonstrated once again that *A. abstrusus* is the dominant species as the lungworm in cats in Türkiye.

INTRODUCTION

Aelurostrongylus abstrusus, *Troglostrongylus brevior*, *T. subcrenatus*, *Oslerus rostratus* (Syn: *Anafilaroides rostratus*) and *Capillaria aerophila* (Syn: *Eucoleus aerophilus*) have been reported as lungworm in domestic cats (Traversa et al., 2009; Jefferies et al., 2010; Brianti et al., 2012; Brianti et al., 2014; Di Cesare et al., 2014; Traversa and Di Cesare, 2016; Giannelli et al., 2017). The infectious stage depends on the species and it is first or third stage larvae. Some species develop directly while others have an indirect development (Doğanay et al., 2018).

Aelurostrongylus abstrusus is a nematode classified in the family Angiostrongylidae of the superfamily Metastrongyloidea (Conboy and Sykes, 2023). It is considered the most important respiratory system parasite of domestic cats in terms of its world and the clinical signs it causes (Traversa and Di Cesare, 2013; Colella et al., 2019). Adult *A. abstrusus* inhabit nodules in the alveoli, alveolar ducts, and bronchioles of infected hosts (Traversa and Di Cesare, 2016). Females are 9-10 mm length and 100 µm width, and males are 5-6 mm length and 70 µm width (Szatmari, 2016). *Aelurostrongylus abstrusus* is a nematode parasite with an indirect life cycle. While the final hosts of the parasite are cats, the intermediate hosts are snails and slugs. Mice, birds, reptiles, and amphibians serve as paratenic hosts for this parasite. Research has suggested that some arthropods, including cockroaches, can be serve as paratenic hosts for *A. abstrusus* (Falsone et al., 2017).

Although nematodes of the genus *Troglostrongylus* were previously considered parasites of wild cats (Traversa and Di Cesare, 2013; Brianti et al., 2014), infections in domestic cats, mostly in young animals, are increasingly reported. In recent years, *T. brevior* has been considered the second most common lungworm of domestic cats after *A. abstrusus*. The adult parasite colonizes the bronchi and bronchioles of infected hosts

(Traversa and Di Cesare, 2016). Adult parasites show sexual dimorphism. Females are 9.6–16.8 mm length and 0.26–0.40 mm width, while males are 6.6–7.2 mm length and 0.2–0.23 mm width (Crisi et al., 2018). The life cycles of *T. brevior* and *A. abstrusus* are similar. In experimental studies, *Helicella barbesiana, H. ustalis, Limax flavus*, *Monaca syriaca*, *Retinella nitellina*, *Theba pisana* and *Helix aspersa* have been reported to act as intermediate hosts for *T. brevior* (Gerichter, 1949; Giannelli et al., 2014; Crisi et al., 2018). *Cornu aspersum* (*H. aspersa*) has also been reported to transmit the agent in natural infections (Morelli et al., 2020). Animals become infected by ingesting the intermediate host containing L3 or mostly paratenic hosts (rodents, frogs, lizards, snakes and birds) (Gerichter, 1949; Anderson, 2000; Bowman et al., 2002). Recent studies suggest that *T. brevior* may be transmitted from infected mothers to their offspring via the galactogenous route (Brianti et al., 2013).

Capillaria aerophila is a nematode in the Trichuridae family that parasitizes the respiratory tracts of cats, dogs, wild carnivores, and rarely humans (Conboy, 2009; Traversa et al., 2011; Khatat et al., 2016). It is considered a low-pathogenic species in cats. Adult parasites are thin, whitish, and filamentous, and are located under the epithelium in the bronchi, bronchioles, and trachea (Bowman et al., 2002; Conboy, 2009). Female parasites measure 16-41 mm in length, while males range from 10-25 mm (Szatmari, 2016). The parasite has a direct life cycle. The eggs that female parasites release after mating are swallowed after coughing and passed into the feces of the infected animal. They reach the infectious stage within 1-2 months in the environment (Bowman et al., 2002). Although it has been reported that earthworms can serve as facultative intermediate or paratenic hosts, it has not been proven that these organisms play a role in the biology of these parasites (Bowman et al., 2002; Conboy, 2009).

The aim of this study is to investigate the prevalence of lungworm in cats in the Kırklareli region according to fecal examination. There are a limited number of studies in Türkiye on the prevalence of lungworm in cats. In the region where the study was conducted, no research has been done on this topic until today, and there is no data on the existence of the parasites. With this study, the first information on the presence and prevalence of lungworm in owned and stray cats in the area has been presented.

MATERIALS AND METHODS

Collection of fecal samples

Within the scope of the study, fecal samples were collected from pet clinics and defecation areas of owned cats in Kırklareli. Permissions for the collection of fecal samples from cats were obtained from the Kırıkkale University Animal Experiments Local Ethics Committee (letter dated 31.01.2022 and numbered E.74224). The 100 cat fecal samples were delivered to the Kırıkkale University, Faculty of Veterinary Medicine, Parasitology Department, Routine and Epidemiology Laboratory under cold chain. Information such as age, breed, gender, whether they showed clinical signs related to respiratory system were recorded. The cats from which the samples were taken were of 12 different breeds (Table 1), 53 were females and 47 were males. While 44 cats were 1 year old and under, 56 were animals over 1 year old.

Fecal analysis

Fecal samples were examined for the presence of eggs and/or first stage larvae of lungworm by using Fülleborn flotation and Baermann method in the laboratory. In order to determine the parasite density in the feces in the samples detected positive for lungworm, the number of eggs and/or larvae per gram (g) of feces was determined using the McMaster method. The Baermann method was performed in accordance with the procedures outlined by Zajac and Conboy (2012), while the Fülleborn flotation and McMaster technique were conducted following the methodology described by Şenlik (2016). Species identification of the detected larvae was carried out following the criteria described by Traversa and Di Cesare (2016) and Morelli et al. (2021). In addition to, the general condition and examination findings of the cats from which the samples were taken were recorded and the number of eggs and/or larvae were compared with the presence and intensity of clinical findings.

Table 1. Number and ratio of animals from which fecal samples were taken according to breed

Breed	Number (n)	Ratio (%)
Crossbred	64	64
British shorthair	10	10
Persian cat	5	5
Bombay cat	5	5
Chinchilla	4	4
Scottish	3	3
Siamese cat	3	3
Ankara cat	2	2
Van cat	1	1
Ragdoll	1	1
Norwegian Forest Cat	1	1
Russian	1	1
Total	**100**	**100**

Statistical analysis

The study results were analyzed using the Chi-Square test in IBM SPSS Statistics 20 program and the results were evaluated at 0.05% confidence interval. Due to low expected cell counts, Fisher's exact test was used to evaluate the association between age and infection status.

RESULTS

During the study, *A. abstrusus* first stage larvae (L1) were detected in eight (8%) of 100 fecal samples examined by the Baermann method, while eggs and/or larvae of other lungworm were not detected (Fig 1). The same feces were subjected to the Fülleborn flotation method, and no larvae and/or eggs of any lung nematode were detected in this method.

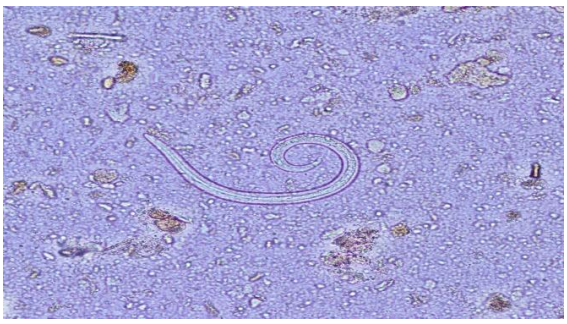

Figure 1. *Aelurostrongylus abstrusus* first stage larvae (L1)

Of the cats in which *A. abstrusus* L1 was detected, one cat was 1 year old or younger (≤1) and seven cats were over 1 year old (>1) (Table 2). However, no significant difference was found between ≤1 year old and >1 year old

cats in terms of *A. abstrusus* L1 positivity (p>0.05). The association between age group and *A. abstrusus* infection status, assessed using the Baermann method, is summarized in Table 3. Infection was detected in 7 of 56 animals older than one year (12.5%), whereas only 1 of 44 animals aged one year or younger (2.3%) tested positive. Fisher's exact test revealed no statistically significant association between age group and infection status (p = 0.075). Although a higher proportion of infections was observed in animals older than one year, this difference did not reach statistical significance.

Of the cats that were positive for *A. abstrusus*, 4 were female and 4 were male. It was determined that 7.5% of the female and 8.5% of the males were infected with *A. abstrusus* (Table 4). There was no statistically significant difference in the presence of *A. abstrusus* between male and female cats (p>0.05).

Table 2. Distribution of *A. abstrusus* presence according to age

			A. abstrusus		Total
			Positive	Negative	
Age	≤ 1	Number	1	43	44
		Age %	2.3	97.7	100.0
		A. abstrusus %	12.5	46.7	44.0
		Total%	1.0	43.0	44.0
	>1	Number	7	49	56
		Age %	12.5	87.5	100.0
		A. abstrusus %	87.5	53.3	56.0
		Total %	7.0	49.0	56.0
Total		Number	8	92	100
		Age %	8.0	92.0	100.0
		A. abtsrusus %	100.0	100.0	100.0
		Total %	8.0	92.0	100.0

Chi-Square: 3.502 p:0.062

Table 3. Association between age group and *A. abstrusus* infection status (Baermann Method)

Age group	**Negative**	***A. abstrusus* positive**	**Total**
> 1 year	49	7	56
≤ 1 year	43	1	44
Total	92	8	100

Table 4. Distribution of *A. abstrusus* presence according to gender

			A. abstrusus		**Total**
			Positive	**Negative**	
Gender	Female	Number	4	49	53
		Gender %	7.5	92.5	100.0
		A. abstrusus %	50.0	53.3	53.0
		Total%	4.0	49.0	53.0
	Male	Number	4	43	47
		Gender %	8.5	91.5	100.0
		A. abstrusus %	50.0	46.7	47.0
		Total %	4.0	43.0	47.0
Total		Number	8	92	100
		Gender %	8.0	92.0	100.0
		A. abstrusus %	100.0	100.0	100.0
		Total %	8.0	92.0	100.0

Chi-Square: 0.31, p:0.573

According to the breeds, six (6%) of the cats found positive for *A. abstrusus* were crossbred, one (1%) was Bombay and one (1%) was Persian. Of the infected cats, 75% were crossbred, 12.5% were Bombay and 12.5% were Persian cats. The infection rate was 9.4% in crossbred cats, 20% in Bombay cats and 20% in Persian cats. However, there was no statistically significant difference between cat breeds in terms of the presence of *A. abstrusus* (p>0.05).

In fecal samples positive for *A. abstrusus*, larval counts per gram, as determined by the McMaster technique, ranged between 50 and 500 larvae (Table 5). Severe respiratory symptoms were observed in three cats in which larval counts of 450 or higher per gram were detected. No clinical signs were observed in the other cats testing positive for *A. abstrusus*.

Table 5 Number of larvae detected per gram of feces in cats infected with *A. abstrusus*

Cat Number	Gender	Age	Breed	Per gram larvae number (n)
1	Male	>1	Crossbred	500
2	Female	>1	Crossbred	500
3	Male	>1	Crossbred	450
4	Male	>1	Crossbred	350
5	Female	>1	Crossbred	350
6	Female	>1	Persian cat	250
7	Female	≤1	Crossbred	100
8	Male	>1	Bombay cat	50

DISCUSSION AND CONCLUSION

While there are many studies in various countries in the world to determine the lungworm in cats, the number of studies conducted in Türkiye to determine the presence and prevalence of these parasites is limited. These studies conducted in Türkiye are generally in the form of case reports (Tuzer et al., 2002; Burgu and Sarımehmetoglu, 2004; Atasever and Yazar, 2009; Gokpinar and Yildiz, 2010; Yildiz et al., 2011; Yildiz and Gokpinar, 2011; Umur et al., 2020), and only two studies were found to determine the prevalence of these parasites (Asılıoglu and Gokpinar, 2021; Yildirim et al., 2023).

In this study, the rate of *A. abstrusus* was determined as 8% in cats in the Kırklareli region according to fecal examination. In previous prevalence studies conducted in Türkiye, the rate of this parasite was determined as 4% in cats in the Kırıkkale and Ankara regions (Asılıoglu and Gokpinar, 2021) and 5% in cats in the Balıkesir region (Yildirim et al., 2023). This rate was found 0.40% in Colombia (Lopez-Osorio et al., 2021), 0.49% in Sweden (Grandi et al., 2017), 0.80% in Switzerland (Giannelli et al., 2017), 0.92% in Belgium (Giannelli et al., 2017), 0.83-17.40% in Portugal (Payo-Puente et al., 2008; Nabais et al., 2014; Waap et al., 2014; Giannelli et al., 2017), 1.70% in the UK (Elsheikha et al., 2019), 1.98% in Brazil (Farago et al., 2022), 1-5% in Spain (Miro et al., 2004; Giannelli et al., 2017), 2.60% in the Netherlands (Robben et al., 2004), 4.34% in France (Giannelli et al., 2017), 2.07% in the USA (Carruth et al., 2019), 5-17.80% in Italy (Traversa et al., 2008; Di Cesare et al., 2015; Giannelli et al., 2015; Giannelli et al., 2017), 6.10% in Romania (Ciopaşiu et al., 2018), 6.60% in Germany (Barutzki and Schaper, 2013), 8% in Greece (Diakou et al., 2015), 8.86-13.60% in Denmark (Olsen et al., 2015; Hansen et al., 2017), 19.8-22.5% in Hungary (Kiszely et al., 2019), 33.3-35.8% in Bulgaria (Stoichev et al., 1982; Giannelli et al., 2017). While the rate of *A. abstrusus* detected in our study was similar to the studies conducted in Greece, Romania, Germany and Denmark, it was lower than in Bulgaria and Hungary, and higher than the studies conducted in Colombia, Switzerland and Belgium. The number of fecal samples examined, the different techniques used in diagnosis, the care and feeding habits of the cats taken as samples, the climate and habitats of the regions where the studies were conducted and the differences in the prevalence of intermediate and/or paratenic hosts are thought to be effective in the emergence of these different results.

When the effect of age on the prevalence of *A. abstrusus* in cats is examined, different results have been obtained in studies conducted worldwide. Although some of these studies reported a statistically significant difference in the presence of *A. abstrusus* in cats according to age groups (Hansen et al., 2017; Carruth et al., 2019), some studies found no significant difference (Asılıoglu and Gokpinar, 2021). Asılıoglu and Gokpinar (2021) reported that they found the rate of *A. abstrusus* to be higher in cats ≤1 year old than in cats >1 year old, but there was no significant difference in the presence of *A. abstrusus* between age groups. Ciopaşiu et al. (2018) reported the rate of *A. abstrusus* to be lower in cats 1-2 years old and older than 2 years old compared to cats between 2 months and 1 year old. Hansen et al. (2017) reported that they encountered this parasite mostly in cats aged 11-51 weeks, and that the positivity rate they detected was statistically significant compared to cats younger than 10 weeks, 1-3 years old, and older than 3 years old. Carruth et al. (2019) found a higher rate of *A. abstrusus* in cats aged 1-12 months compared to cats older than 12 months and found that there was a significant difference between age groups in terms of the presence of this parasite. In a study conducted by Giannelli et al. (2017) in various European countries, the rate of *A. abstrusus* was found to be higher in cats older than 2 years of age compared to cats <6 months, 6-12 months and 1-2 years old. In the present study, 7 of the 8 cats positive for *A. abstrusus* were >1 year old, while one was ≤1 year old. However, no significant difference was found between age groups in terms of the presence of *A. abstrusus*. Contrary to most of the other studies evaluating according to age, in this study, higher levels of *A. abstrusus* were found in adult cats compared to young cats. The reason why this agent was found more in cats older than one year is thought to be due to the fact that the number of samples taken in this study was higher in this age group and that these animals had more encounters with the parasite's intermediate or paratenic hosts at some point in their lives.

In the present study, 7.5% of female cats and 8.5% of male cats whose feces were examined were found to be positive for *A. abstrusus* L1. However, there was no significant difference between males and females in terms of the presence of this parasite ($p>0.05$). Similar to this study, Carruth et al. (2019), and Asılıoglu and Gokpinar (2021) found the rate of *A. abstrusus* to be higher in male cats than in females, but reported that there was no statistically significant difference between the two gender in terms of the presence of this parasite. Hansen et al. (2017) found that the rate of *A. abstrusus* was higher in females than in males, although there was no statistically significant difference between the gender. Elsheikha et al. (2019) determined that there was no significant difference between the gender in their study in England. When the study results were evaluated, the results of the studies conducted to date show that there is no gender predisposition for *A. abstrusus* in cats.

In this study, six of the eight cats in which *A. abstrusus* L1 were detected were crossbreds (75%), one was Bombay (12.5%), and one was Persian (12.5%). Although the infection rate in crossbred cats was higher than in other breeds, no statistically significant difference was found between cat breeds in terms of the presence of *A. abstrusus*. The high number of infected crossbred cats is

due to the fact that most of the sampled cats (64%) were crossbred. Asılıoglu and Gokpinar (2021) evaluated the cats they sampled as crossbreds and purebreds and detected a higher rate of *A. abstrusus* in crossbred cats than in purebreds. However, the researchers reported that there was no significant difference between crossbred and purebred cats in terms of the presence of this parasite.

The McMaster technique was applied to determine the number of larvae per gram in stool samples that tested positive for *A. abstrusus*. Accordingly, the number of larvae per gram of feces was determined as minimum 50 and maximum 500. To date, no study has been found to determine the number of larvae per gram of feces in cats infected with *A. abstrusus*. In the present study, respiratory symptoms were observed in three infected cats with fecal larval counts of ≥450 larvae per gram, suggesting a potential association between pulmonary parasite burden and clinical condition. Nevertheless, further investigations involving a larger number of cats infected with *A. abstrusus* are required to better clarify the relationship between lung parasite load and the severity of clinical signs.

To date, *T. brevior* has not been detected in fecal examinations of domestic cats in Türkiye. Umur et al. (2020) reported that they *T. brevior* was detected in the lung tissue of two domestic cats necropsied. In studies conducted in various countries, the rate of *T. brevior* was found to be 1.2-14% in domestic cats. No larvae of this parasite were found in any of the fecal samples examined in this study. In order to determine the prevalence of this parasite in Türkiye, we believe that more studies should be conducted and more cat feces should be examined.

In this study, *C. aerophila* eggs were not found in the examined cat feces. In previous studies conducted in Türkiye, the *C. aerophila* rate was determined as 3.3% in Ankara (Mimioğlu, 1951) and 4% in Elazığ (Altaş and Taşan, 1999). In studies conducted worldwide, *C. aerophila* was detected at an average rate of 6.6% in seven different countries of Europe (Rehbein et al., 2014), 11% in Italy (Traversa et al., 2009), and 8.3% in Serbia (Iliç, 2009). We believe that the fact that all of the cats from which fecal samples were taken in this study were owned cats and had little contact with the environment, and that the study had different climatic conditions compared to the regions in Türkiye where the agent was detected, were effective in obtaining this result.

In conclusion, *A. abstrusus* was detected for the first time in cats in Kırklareli region. *T. brevior* and *C. aerophila* were not found in cats in the region. As in previous studies, it has been demonstrated once again that *A. abstrusus* is the dominant species of lungworm in cats in Türkiye. It is thought that *A. abstrusus* should also be taken into consideration in cats with respiratory system symptoms. We believe that more studies should be conducted to determine lungworms in cats both in the region and in Türkiye.

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This study is summarized from Barış Aşılıoğlu's master's thesis

Ethical Declaration

Permissions for the collection of fecal samples from cats were obtained from the Kırıkkale University Animal Experiments Local Ethics Committee (letter dated 31.01.2022 and numbered E.74224).

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: B.A., S.G., Design: S.G., B.A., Data Collection or Processing: S.G., B.A., Analysis or Interpretation: B.A., S.G., Literature Search: B.A., S.G., Writing: S.G., B.A.

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