Respiratory nematodes in cat populations of Italy

Angela Di Cesare[1], Fabrizia Veronesi[2], Eleonora Grillotti[1,3], Simone Manzocchi[4], Stefania Perrucci[5], Paola Beraldo[6], Stefania Cazzin[7], Claudio De Liberato[8], Luciano A. Barros[1,9], Giulia Simonato[10], Donato Traversa[1*]

1. Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy;

2. Department of Veterinary Medicine, University of Perugia,Perugia, Italy;

3. Ambulatorio Veterinario Centro Italia, Rieti, Italy;

4. Novara Day Lab - IDEXX Laboratories, Monticello, Italy;

5. Department of Veterinary Science, University of Pisa, Pisa, Italy;

6. Division of Veterinary Pathology (DIAL), University of Udine, Udine, Italy;

7. Istituto Zooprofilattico delle Venezie, Legnaro, Padova, Italy;

8. Istituto Zooprofilattico Sperimentale del Lazio e della Toscana BM. Aleandri, Rome, Italy;

9. Facultade de Medicina Veterinaria MSV/UFF Niteroi, Niteroi, RJ, Brazil;

10. Department of Animal Medicine, Production and Health, University of Padua, Padua, Italy.

Corresponding author: Donato Traversa, E-mail: dtraversa@unite.it

Abstract

The occurrence of common respiratory parasites of domestic cats (the metastrongyloid cat lungworm *Aelurostrongylus abstrusus* and the trichuroid *Capillaria aerophila*) and of neglected respiratory nematodes of felids (*Troglostrongylus brevior, Angiostrongylus chabaudi* and *Oslerus rostratus*) was here evaluated in two and three geographical sites of Northern and Central Italy, respectively. In 2014–2015, individual fecal samples of 868 domestic cats were examined microscopically and genetically, and epidemiological data related to parasitic infections were evaluated as possible risk factors by binary logistic regression models. The most common parasite was *A. abstrusus* in both mono- and poli-specific infections, followed by *T. brevior* and *C. aerophila*, while cats scored negative for other parasites. Cats positive for *A. abstrusus* (1.9–17 % infection rate) and *C. aerophila* (0.9–4.8 % infection rate) were found in all examined sites, while cats scored positive for *T. brevior* (1–14.3 % infection rate) in four sites. Also, *T. brevior* was here found for the first time in a domestic cat from a mountainous area of Northern Italy. The occurrence of lungworms was statistically related to the presence of respiratory signs and more significant in cats with mixed infection by other lungworms and/or intestinal parasites. Cats living in site C of Central Italy resulted statistically more at risk of infection for lungworms than cats living in the other study sites, while animals ageing less than 1 year were at more risk for troglostrongylosis. Finally, the presence of lungworms was more significant in cats with mixed infection by other lungworms and/or intestinal parasites. These results are discussed under epidemiological and clinical points of views.

Keywords: Aelurostrongylus abstrusus; Troglostrongylus brevior; Capillaria aerophile; Lungworms; Italy; Diagnosis; Epidemiology; Risk factors.

Introduction

Parasitic nematodes of the respiratory airways of domestic cats (*Felis silvestris catus*) have been traditionally considered to be the metastrongyloid *Aelurostrongylus abstrusus* (the cat lungworm) and the trichuroid *Capillaria aerophila* syn. *Eucoleus aerophilus* (Traversa and Di Cesare 2013). Specifically, *A. abstrusus* is the most significant respiratory parasite of cats in terms of geographic distribution and practical relevance, while the trichuroid nematode *C. aerophila* has been little recognized until recently as an important respiratory pathogen of cats, despite it has been found since a long time in the trachea and bronchi of wild and domestic carnivores (including cats) and, sometimes, people (Traversa and Di Cesare 2013).

In the past few years, other metastrongyloids have been described from *F. s. catus* either after a long time since the first description, or for the first time. *Troglostrongylus brevior* was first found last century in a *Felis silvestris lybica*, the African wildcat (formerly *Felis ocreata*), and in a *Felis chaus*, the jungle cat (formerly *Catolynx chaus*) from Palestine (Gerichter 1949). After 10 years, the nematode was recorded in a European wildcat (*Felis silvestris silvestris*) and in an animal not well defined as a feral cat or a wildcat from Central Italy (Paggi 1959). Since then, *T. brevior* has not been reported until 2010, when the first inequivocal description from domestic cats has been published from Ibiza island of Spain (Jefferies et al. 2010). Thereafter, *T. brevior* has been increasingly described in other European islands (Brianti et al. 2012; Tamponi et al. 2014; Diakou et al. 2014) and in mountainous sub-Apennine regions of Central and Southern Italy (Brianti et al. 2013; Di Cesare et al. 2014a, 2015a). Although *Oslerus rostratus* was described for the first time in *F. s. catus* (Gerichter 1949), this nematode has been then reported in bobcats (Watson et al. 1981) and the domestic cat has been considered an accidental host (Juste et al. 1992). At the moment, *O. rostratus* has only been recorded in cats from confined areas such as Sri Lanka, Maiorca, and Hawaii and, more recently, in two single cats, again from Italian islands (Brianti et al. 2014a; Varcasia et al. 2015). The metastrongyloid *Angiostrongylus chabaudi* was described for the first time, last century from heart and lung arteries of European wildcats coming from an area of Central Italy where hundreds of other animals, i.e., domestic cats, dogs, foxes, and badgers, were negative for this parasite at necroscopic examination (Biocca 1957). In the past 2 years, immature adults of this parasite have been recovered at the necropsy of two domestic cats from Sardinia island of Italy (Varcasia et al. 2014) and Central Italy (Traversa et al. 2015), respectively.

These recent data suggest that regions of Southern Europe, and particularly Italy, may offer suitable biological and epidemiological conditions for the occurrence and expansion of felid (cardio-)respiratory parasites. Nonetheless, in the past decade, only few and limited studies have been performed to investigate their presence in cat populations living in Italy (Traversa et al. 2008a, b; Di Cesare et al. 2011; Tamponi et al. 2014). There are no recent information on continental territories of this country that apparently offers conditions suitable for the occurrence and the expansion of these parasites.

Additionally, there is a merit in investigating for the first time and on large numbers the risk factors for the (re)discovered respiratory felid parasites, especially *T. brevior*. Therefore, the present investigation aimed to enhance our knowledge on distribution and risk factors of (cardio-)respiratory parasitic infections in different cat populations of continental Italy.

Materials and methods

1. Sampling and copromicroscopic examinations

Overall, 868 cats were randomly examined from five Italian sites, i.e., two from the North (Friuli Venezia Giulia region, site A, n. 104 cats; Piedmont region, site B, n. 106 cats) and three from the Center (Abruzzo-Molise regions, site C, n. 182 cats; Latium region, site D, n. 207 cats, Umbria-Toscana regions, site E, n. 269 cats) of Italy. The complete anamnesis (i.e., sex, age, living environment, presence/absence of respiratory signs, other clinical signs) was recorded for each animal.

Animals were enrolled when referred for medical problems or for routine parasitological examination, either to practitioners or to University Teaching Veterinary Hospitals. Fecal samples were subjected to a copro-microscopic examination consisting of standard floatation procedures and to the Baermann method. Specifically, samples were undertaken to standard flotation methods with a sugar solution with a 1.200 specific gravity (s.g.) and a 1.350 s.g. zinc sulfate solution. The Baermann technique was performed according to the standard protocol. All parasites recovered were identified according to existing descriptions and keys (Sloss et al. 1994; Traversa et al. 2011; Traversa and Di Cesare 2013; Brianti et al. 2014b).

2. Molecular examinations

All samples positive for at least one trichuroid egg or a metastrongyloid larva were subjected to molecular assays to confirm the identity of parasitic elements retrieved. The samples were examined as previously described with a semi-nested PCR able to unequivocally identify the eggs of *C. aerophila* (Di Cesare et al. 2012) and with a multiplex PCR that simultaneouslyidentifies and discriminates *A. abstrusus*, *T. brevior*, and *A. chabaudi* (Di Cesare et al. 2015b). Selected amplicons were sequenced as previously described (Di Cesare et al. 2014a, 2015b).

3. Statistical analysis

The differences in prevalence of each respiratory parasite retrieved according to epidemiological data were evaluated using chi-squared test. The individual cat data (i.e., independent variables) were analyzed for risk factor analysis using binary logistic regression models, where single infections by either *T. brevior* or *A. abstrusus*, and total lungworm (including *C. aerophila*) occurrence were the dependent variables (negative=0; positive=1). Due to the low number of positive samples, single infections by *C. aerophila* were not included in the risk factor analysis. The independent variables offered to the models and their categorization are listed in Table 2. A matrix correlation of the estimates was also produced in order to exclude variables that could possibly be highly correlated with each other.

For *T. brevior*, the variable provenance had a 0 count in site B (i.e., no positive cats in this region); thus, odds ratio could not be calculated. As difference in prevalence in the sites examined resulted highly significant ($p<0.01$) in the univariate screening by the chi-squared test, the variable provenance was used in the logistic models based on the so-called the rule of three (Hanley and Lippmann-Hand 1983). Specifically, three cat positive for *T. brevior* were randomly assigned to Piedmont region.

All statistical analyses were performed using SPSS software, version 13.0 for Windows.

Results

The prevalence of each lungworm is reported in Tables 1 and 2 according to single and mixed infections in examined sites and epidemiological data, respectively. The most common parasite was *A. abstrusus* in both mono and poli-specific infections, followed by *T. brevior* and *C. aerophila* (Table

1). No cats scored positive for *A. chabaudi* or *O. rostratus*. Single infections were more frequent than mixed infections for all the three lungworms found (Table 1). The highest prevalence rate for both monospecific infections by *A. abstrusus*, *T. brevior*, and *C. aerophila*, and simultaneous infections by metastrongyloids was recorded in site C. The most common coinfection in the five sites examined was that by *A. abstrusus* and *T. brevior* (Table 1). Cats positive for *A. abstrusus* and *C. aerophila* were found in all examined sites, with an overall infection rate ranging from 1.9%(site A) to 17%(site C), and from 0.9%(site B) to 4.8 % (site A), respectively (Table 2). The overall infection rate by *T. brevior* ranged from 1 % (site A) to 14.3 % (site C), with no infected cats found in site B (Table 2).

The molecular procedures confirmed the identity of the eggs (i.e., *C. aerophila*) and larvae (i.e., *A. abstrusus* and *T. brevior*) found in positive cats. No samples scored genetically positive for *A. chabaudi*. The statistical analysis showed that the presence of lungworms is significant higher in (i) cats from site C, (ii) cats less than 1-year-old cats for *T. brevior*, (iii) cats showing respiratory signs, and (iv) cats with mixed infection by lungworms and/or intestinal parasites (Table 3). The multivariate analysis confirmed that risk factors were the variables above (Table 3) excluding for the coinfection with other lungworms.

Discussions

The present study documents that *A. abstrusus* is endemic in all territories examined, even with high infection rates, with the exception of site A (Tables 1 and 2). Until a decade ago, *A. abstrusus* was considered sporadic and most records were either single clinical cases or the accidental finding of larvae in faeces from subclinically infected cats (Traversa and Guglielmini 2008). Nevertheless, in the past few years, cat aelurostrongylosis has been described from many countries, including Italy, with apparently increasing rates of detection (Traversa et al. 2010; Traversa and Di Cesare 2013, 2014; Barutzki and Schaper 2013). This possible spread of *A. abstrusus*, along with the recent records of neglected or unusual (cardio)respiratory parasites, has raised the question as to whether these nematodes are now emerging in some geographic areas or the rise in documented diagnosis is due to increased awareness in the scientific community and among veterinarians (Traversa and Di Cesare 2013, 2014; Otranto et al. 2013; Traversa 2014).

The occurrence of *A. abstrusus* in site B confirms its presence in this part of Northern Italy that was documented in past clinical studies in the confining Lombardy region (Grandi et al. 2005). Until now, there were no published cases of cat aelurostrongylosis in north-eastern areas of Italy (e.g., site A), despite this nematode was recently found in few cats (Antonio Frangipane di Regalbono, unpublished). Given that few cats were found to harbor *A. abstrusus* in this study, it can be argued that *A. abstrusus*, and especially *T. brevior* (see below), are relatively rare in populations of domestic cats of North-Eastern Italy. This scenario is in contrast with the findings obtained in mountainous territories of Central Italy by recent surveys and by the present study. In fact, the present data are in accordance with the high infection rates by *A. abstrusus* (i.e., up to ~25 %) recorded few years ago in sites C and D (Traversa et al. 2008a, b). Interestingly, the rates of aelurostrongylosis for site E are higher than rates obtained from a portion of the same areas few years ago (Riggio et al. 2013).

The finding of a single infected cat from site A represents the first report of *T. brevior* in the North of Italy, as in this country, this parasite was found, thus far, only in Sicily and Sardinia islands, and in Central and southern sub-Apennine regions (Brianti et al. 2012, 2013; Di Cesare et al. 2014a, 2015a; Tamponi et al. 2014; Traversa et al. 2014, 2015). The data obtained show that *T. brevior* is endemic in mountainous sub-Apennine territories of Central Italy, where it may now occur in domestic cats with rates equivalent to those of *A. abstrusus* (Tables 1 and 2). It is worthy of note that a recent retrospective study has documented that, in the same areas, the occurrence of *T. brevior* in cats with lungworm infections was negligible until few years ago (Di Cesare et al. 2015c). Thus, it is likely that in the past few years, some ecological drivers have promoted these epidemiological modifications.

The presence of *C. aerophila* in all sites examined, although with infection rates inferior to those recorded for metastrongyloids (Tables 1 and 2), confirms that this nematode may infect domestic cats wherever ecological conditions are suitable for its development and circulation among animal populations. It is noteworthy that most positive cats harbored the haplotype 1 of *C. aerophila*, which is the most common in terms of geographic distribution in Europe and is shared by wildlife (e.g., foxes, wildcats) and companion animals, including *F. s. catus* (Di Cesare et al. 2014b; Otranto et al. 2015; Veronesi et al. 2015).

Morphological and morphometric features of larval *A. chabaudi* are not known (Traversa et al. 2015), but no positive samples contained first stage larvae with *Angiostrongylus*-like characteristics. The absence of cats positive for *A. chabaudi*, even in sites of Central Italy, where this nematode has been documented in wildcats for the first time (Biocca 1957) and, very recently, in a domestic cat with a mixed infection by *A. abstrusus* and *T. brevior* (Traversa et al. 2015) further indicates that it is likely confined in its limited ecological niches and that at the moment domestic cats should be considered occasional and unsuitable hosts (Di Cesare et al. 2015d). Analogously, all cats were negative for *O. rostratus*. The domestic cat has been considered an accidental host for this nematode (Juste et al. 1992) that has been recorded only in domestic cats living in confined areas (Traversa and Di Cesare 2013). Accordingly, the most recent cases of cat oslerosis regard two domestic cats from Sardinia (Varcasia et al. 2015) and Sicily (Brianti et al. 2014a) islands.

Overall, this scenario supports the hypothesis that some drivers may have recently promoted an expansion of *A. abstrusus* and an overflow of *T. brevior* from wild reservoirs (e.g., European wildcats) to domestic hosts (Traversa and Di Cesare 2013; Traversa et al. 2015; Di Cesare et al. 2015d). In fact, *T. brevior* has been found thus far only in domestic cats living in regions where populations of *Felis s. silvestris* are present (Traversa and Di Cesare 2013, 2014; Otranto et al. 2015; Traversa et al. 2015; Di Cesare et al. 2015d) and are infected with this parasite even with high rates (Beraldo et al. 2014; Falsone et al. 2014; Veronesi et al. 2015).

The present findings corroborate this possibility, as in Northern Italy *T. brevior* has been found only in site A, where *T. brevior*-infected *F. s. silvestris* are present (Beraldo et al., 2014) and not in site B, where wildcats are considered nowadays absent. Additionally, the high occurrence of *A. abstrusus* in wildcat populations of Central Italy (Veronesi et al. 2015), in contrast with surveys from the south (Falsone et al. 2014) and the north (Beraldo et al. 2014) of the country, suggests that in some territories, the same drivers promoting bridging infections from wildlife to domestic cats have the potential to foster the exchange of lungworms in both ways.

Accordingly, the statistical analysis demonstrated a significant presence of *A. abstrusus* and *T. brevior* in site C of Central Italy, meaning that cats living in this area are at higher risk of lungworm infections than animals living in the other study sites.

Hence, it can be argued that some geographic regions offer suitable factors for the current epidemiological modifications. In fact, *T. brevior* and *A. chabaudi* occur in wildcats of countries of Central Europe, e.g., Germany (Steeb et al. 2014) despite, at the moment, there is no documented case of these parasites in domestic cats from the same countries. Analogously, *A. abstrusus* was not recently recorded in wildcats of Germany (Krone et al. 2008).

The existence of common patterns of transmission of lungworms between wildlife and domestic cats is here confirmed by the fact that the presence of a given respiratory parasite is not a factor that predispose to the infection with other lungworms (Tables 2 and 3), but cats positive for different lungworms are rather subjected to the same risk factors. Furthermore, until now, mixed infections by *A. abstrusus* and other parasites were more frequent than monospecific parasitic infections by rare metastrongyloids (Di Cesare et al. 2015d), although, as here demonstrated, *T. brevior* can frequently occur in mono-specific infections (Table 1).

The Sardinia island of Italy is another region where epidemiological conditions could influence a similar expansion of lungworms in felid populations. In fact, *T. brevior* occurs frequently in domestic cats of this Island, with rates similar to those of Central Italy (Tamponi et al. 2014). Also, the other only record of *A. chabaudi* in a domestic cat is from Sardinia (Varcasia et al. 2014), where also O. rostratus has been recently recorded in a domestic cat with a mixed infection by *A. abstrusus, T. brevior* and *C. aerophila* (Varcasia et al2015). The populations of African wildcat living in this territory could be a likely source of infection for domestic cats with these rare parasites, as for instance, this felid is a natural host of *T. brevior* (Gerichter 1949). Unfortunately, at the moment there is no data on the presence of (cardio-)respiratory nematodes in African wildcats of Sardinia.

Under a practical point of view, the statistical analysis confirmed that both young and adult cats may be infected by *A. abstrusus*, and that the age of examined animals is inconstantly correlated with the presence of this parasite (Tables 2 and 3), as shown in previous surveys (Traversa et al. 2008a; Mircean et al. 2010; Knaus et al. 2011; Capári et al. 2013).

Conversely, age is here ultimately confirmed as a risk factor for the infection caused by *T. brevior*. In fact, this parasite occurred mostly in kittens younger than 1 year and in 1–2 years old cats, i.e., 70 and 20 % of the here positive cats (Tables 2 and 3). Such an epidemiological feature can be explained by a possible vertical transmission of the parasite (Brianti et al. 2013). Also, it is likely that kittens and young cats are more prone than adult cats to acquire the infection by *T. brevior* for a higher susceptibility to a lungworm that usually infect other animals (Traversa and Di Cesare 2013).

All lungworms here found confirmed to be of importance under a clinical standpoint, as their presence was significantly correlated to respiratory clinical signs (Table 3). Cat lungworm infections have been often erroneously considered subclinical or self-limiting (Traversa and Guglielmini 2008). Although apparently healthy cats may be infected by *A. abstrusus* and/or *C. aerophila* (Traversa et al. 2008a, b, 2009), the present results demonstrate that there is a statistical correlation between aelurostrongylosis or lung capillariosis and respiratory signs, as also previously evidenced (Traversa et al. 2008a, 2009). It is here ultimately confirmed that also troglostrongylosis is significantly associated to the presence of respiratory signs. This is of importance if one considers that this lungworm mostly infects young animals, in which the infection is often severe and sometimes fatal (Brianti et al. 2012, 2013; Traversa and Di Cesare 2013).

In conclusion, this study showed that different respiratory nematodes are present in cats of Italy, even with high infection rates. Given the impact that these parasites may have for the health and welfare of domestic cats, the possible geographic expansion of the cat lungworm *A. abstrusus* and of *C. aerophila*, and the spill-over of *T. brevior* from wild reservoirs to domestic hosts, is strongly advisable that these nematodes should be included into the differential diagnosis of respiratory diseases of cats. Also, further epidemiological surveys are warranted to understand whether and where *A. chabaudi* and *O. rostratus* may be harbored by domestic cats and whether should be considered as pathogens truly causing (cardio-)respiratory diseases in domestic hosts.

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Conflict of interests

This study has not been financed not influenced by any third party. ADC holds a post-doc fellowship on parasites different than feline lungworms and she has participated in the present study in the framework of her freedom of scientific research. The authors declare that there are no conflicts of interest.

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Table 1. Number of cats examined for lungworms broken down according to five Italian sites examined and their positivity to single and mixed infections.

Site/Cats	Aab/%	Tb/%	Cae/%	Aab+Tbr/%	Aab+Cae/%	Tbr+Cae/%	Aab+Tbr+Cae/%
A/104	1/0.9	0/0	3/2.9	0/0	1/0.9	1/0.9	0/0
B/106	11/10.4	0/0	0/0	0/0	1/0.9	0/0	0/0
C/182	18/9.9	14/7.7	2/1.1	9/4.9	1/0.5	0/0	3/1.6
D/207	13/6.3	6/2.9	4/1.9	2/0.9	0/0	0/0	0/0
E/269	9/3.3	13/4.8	7/2.6	2/0.7	0/0	0/0	0/0
Total/868	52/6	33/3.8	16/1.8	13/1.5	3/0.3	1/0.1	3/0.3

Sites: A, Friuli Venezia Giulia; B, Piedmont; C, Abruzzo-Molise; D, Latium; E, Umbria-Tuscany

Aab Aelurostrongylus abstrusus, *Tbr Troglostrongylus brevior*, *Cae Capillaria aerophila*

Table 2. Number of cats examined, positivity (pos), and overall prevalence of lungworms according to epidemiological data, and results of the univariate analysis (chi-squared test).

		Aab		*Tbr*		*Cae*	
Factors	Examined	pos (%)	$\chi^2 - p$	pos (%)	$\chi^2 - p$	pos (%)	$\chi^2 - p$
Site							
A	104	2 (1.9)	32.039	1 (0.9)	36.346	5 (4.8)	ns
B	106	12 (11.3)	p<0.01	0	p<0.01	1 (0.9)	
C	182	31 (17)		26 (14.3)		6 (3.3)	
D	207	15 (7.2)		8 (3.9)		4 (1.9)	
E	269	11 (4.1)		15 (5.6)		7 (2.6)	
Age (months)							
<12	281	32 (11.4)	8.263	35 (12.5)	35.543	8 (2.8)	ns
12–24	288	24 (8.3)	p<0.05	10 (3.5)	p<0.01	9 (3.1)	
24–36	110	7 (6.4)		3 (2.7)		0	
>36	189	8 (4.2)		2 (1.1)		6 (3.2)	
Sex							
Female	436	34 (7.8)	ns	28 (6.4)	ns	11 (2.5)	ns
Male	429	37 (8.6)		22 (5.1)		12 (2.8)	
Outside habitat							
No	54	3 (5.6)	ns	3 (5.6)	ns	0	ns
Yes	814	68 (8.4)		47 (5.8)		23 (2.8)	
Respiratory signs							
No	704	33 (4.7)	58.067	21 (3.0)	52.948	14 (2.0)	6,314
Yes	164	38 (23.2)	p<0.01	29 (17.7)	p<0.01	9 (5.5)	p<0.05
Other signs							
No	684	55 (8.0)	ns	34 (5.0)	ns	16 (2.3)	ns
Yes	184	16 (8.7)		16 (8.7)		7 (3.8)	
Intestinal parasites							
No	692	37 (5.3)	34,386	30 (4.3)	12,238	12 (1.7)	12,033
Yes	168	32 (19)	p<0.01	19 (11.3)	p<0.01	11 (6.5)	p<0.01
Aab							
No	797	–	–	34 (4.3)	40,082	17 (2.1)	10,087
Yes	71	–	–	16 (22.5)	0<0.01	6 (8.5)	p<0.01
Tbr							
No	818	55 (6.7)	40.082	–	–	19 (2.3)	5.888
Yes	50	16 (32)	0<0.01	–	–	4 (8)	p<0.05
Cae							
No	845	65 (7.7)	10.087	46 (5.4)	5.888	–	–
Yes	23	62 (6.1)	p<0.01	41 (7.4)	p<0.05	–	–

Sites: A, Friuli Venezia Giulia; B, Piedmont; C, Abruzzo-Molise; D, Latium; E, Umbria-Tuscany

Aab Aelurostrongylus abstrusus, *Tbr Troglostrongylus brevior*, *Cae Capillaria aerophila*, *ns* not significant

Table 3. Significant risk factors (odds ratio=OR; p<0.01) associated with *Aelurostrongylus abstrusus* (Aab), *Troglostrongylus brevior* (Tbr), and with presence of lungworms in general (including *Capillaria aerophila*, Cae)

Independent variables	*Aab* OR (95 % CI)	*Tbr* OR (95 % CI)	Lungworms OR (95 % CI)
Site C	2.64 (1.54–4.54)	3.68 (1.88–7.19)	2.75 (1.81–4.16)
<12 months	ns	3.61 (1.98–6.57)	1.82 (1.268–2.62)
Respiratory signs	2.29 (1.71–3.07)	2.65 (1.86–3.79)	2.60 (2.06–3.31)
Intestinal parasites	1.87 (1.38–2.55)	ns	1.85 (1.44–2.39)

ns not significant