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Clinical efficacy of a parasiticide formulation containing eprinomectin, esafoxolaner and praziquantel (NexGard® Combo) in the treatment of natural feline aelurostrongylosis and troglostrongylosis

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ABSTRACT

The metastrongyloids *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* are primary causes of feline clinical respiratory disease. The present field trial evaluated the clinical efficacy of a spot-on formulation containing eprinomectin, esafoxolaner and praziquantel (NexGard® Combo) administered per label recommendations to cats affected with aelurostrongylosis and/or troglostrongylosis. Overall, 36 naturally infected cats were randomly assigned to Group 1 (G1) or Group 2 (G2) of 18 cats each. The two groups included 6 cats with *A. abstrusus*, *T. brevior* and mixed infection, each. All cats completed the study. Cats in G1 were treated on study Days (SDs) 0 and 28±2. Cats in G2 served as negative control until SD 56±2 and were then treated on SD 56±2 and 84±2. On SD 0/-7, 28±2 and SD 56±2 all cats were subjected to parasitological (quali-quantitative Baermann) and clinical examinations (physical exams and thoracic X-rays). Hematology and biochemistry analyses were performed on SD 0/-7 and SD 56±2. On SD 84±2 quali-quantitative Baermann, clinical examination and thorax radiography were performed on all G2 cats and on two G1 cats that still had radiographic alterations on SD 56±2. On SD 112±2 all G2 cats were subjected to parasitological and clinical evaluations and one cat from G1 that still had radiographic signs at SD 84±2 was clinically and radiographically evaluated. Efficacy criteria were the reduction of larval shedding in faeces and the clinical response in terms of pathological and radiographic scores after treatment compared to the baseline. An efficacy of 100 % based on LPG reduction was recorded after one (20/24 cats) or two (all 24 cats) treatments in cats with single infection by *A. abstrusus* or *T. brevior*. For cats with mixed infections, larval shedding was stopped after one (11/12 cats) or two (all 12 cats) treatments. Statistically significant clinical and radiographic improvement was evident in all study cats after 2 treatments. The present data show that two monthly treatments with NexGard® Combo stopped larval shedding and led to a significant clinical recovery and a complete resolution of radiographic abnormalities in cats infected with *A. abstrusus* and/or *T. brevior*.

1. Introduction

Respiratory diseases caused by the parasitic nematodes *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* pose a significant threat to the health and welfare of domestic cats. The life cycle of *A. abstrusus* and *T. brevior* is indirect. Cats become infected by ingesting infective third-stage larvae (L3) harbored by molluscs acting as intermediate hosts or by preying on paratenic hosts, e.g. birds, rodents and small reptiles (Traversa et al., 2010; Morelli et al., 2021). Data obtained in natural conditions strongly suggest that *T. brevior* is transmitted vertically from the queen to the litter *via* the milk (Brianti et al., 2013; Traversa et al., 2018).

Aelurostrongylus abstrusus is distributed worldwide, while *T. brevior* has been thus far recorded in Europe and in the Middle East (reviewed

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Table 1
Procedures performed at each timepoint of the study for Group 1 (G1) and Group 2 (G2).

Study Day	Quali-quantitative Baermann	PCR	Randomization to G1 and G2	Clinical examination	Thorax radiography	Blood sampling	Treatment	Study completion
−7–0	x	x	x	x	x	x		
0							G1	
28±2	x			x	x		G1	
56±2	x			x	x	x	G2	G1*
84±2	G2			G2**	G2**		G2	
112±2	G2			G2** ***	G2** ***	G2		G2*
140, 168 ±2				***	***			
196, 224 ±2								

x: both groups; G1: 18 cats treated with NexGard® Combo at Study Days 0 and 28 ±2; G2: 18 cats treated with NexGard® Combo at Study Days 56 ±2 and 84±2. *except cats showing pulmonary radiographic alterations; **and remaining G1 cats; ***any remaining cat showing pulmonary radiographic alterations.

by Barutzki and Schaper, 2013; reviewed by Elsheikha et al., 2016; reviewed by Traversa et al., 2021).

Adult stages of *A. abstrusus* and *T. brevior* reside in the lung parenchyma, alveoli and alveolar ducts, and in bronchioles and bronchi, respectively. Aelurostrongylosis is characterized by variable clinical features ranging from pulmonary lesions with mild clinical signs to evident signs like sneezing, ocular and nasal discharge, coughing, dyspnea, and tachypnea (Schnyder et al., 2014; Crisi et al., 2017; Febo et al., 2019; Napoli et al., 2023). Life-threatening conditions, such as pleural effusion and pneumothorax with respiratory failure, neurological signs due to hemorrhagic meningoencephalomyelitis or fatal pulmonary hypertension have also been described (Crisi et al., 2017; Vezzosi et al., 2020; Tinoco et al., 2022).

Troglostrongylus brevior is usually more pathogenic in young animals than in adult cats in which the clinical relevance of the disease is limited (Traversa and Di Cesare, 2016; Crisi et al., 2018). The pathogenicity in young animals may be due to its larger size and its location in the upper respiratory tract whereas *Aelurostrongylus* worms are located deeper, in alveoli. Infected cats show signs that overlap those of aelurostrongylosis, though they usually are more severe and most often fatal in kittens and young cats (Diakou et al., 2014; Traversa et al., 2014; Crisi et al., 2018).

The correlation between the severity of the clinical presentation and pulmonary damages detectable at thoracic X-rays of cats infected with *A. abstrusus* and *T. brevior* is only partial. In fact, cats with no clinical signs can have lung lesions and may sooner or later develop a manifest disease, and cats with overt disease may show varying degrees of pulmonary alterations (Crisi et al., 2017; Febo et al., 2019; Morelli et al., 2021). Mixed infections are often observed in enzootic areas and lead to more complex clinical and radiographic presentations compared to monospecific parasitosis (Febo et al., 2019).

Different parasiticides (e.g. eprinomectin, moxidectin, emodepside) are labelled for treating aelurostrongylosis and/or troglostrongylosis and their efficacy has been evaluated based mainly on the reduction of larval shedding in faeces and of worm counts at necropsy of experimentally infected cats. Conversely, data on the clinical efficacy of parasiticides against felid lungworms are limited. The present study investigated the efficacy of a topical formulation containing eprinomectin, esafoxolaner and praziquantel (NexGard® Combo, Boehringer Ingelheim) against infections caused by *A. abstrusus* and/or *T. brevior* by evaluating and comparing clinical, laboratory and radiographic alterations before and after treatment of naturally infected cats.

2. Materials and methods

2.1. Study animal and design

The study had a randomized, negative controlled, blinded design and was conducted in three different endemic areas of central Italy. Approval to conduct the study was obtained from the Italian regulatory authority (Italian Health Ministry, authorization no. 00023176–07/10/2021-DGSAF-MDS-A) and by the Ethics Committee of the Department of Veterinary Medicine of the University of Teramo (no. 6112 del 28/02/2022).

A total of 36 privately owned domestic cats with natural *A. abstrusus* and/or *T. brevior* infection diagnosed at the Baermann's test (Sloss et al., 1994), and without any other concomitant diseases, were recruited. The study cats were diagnosed with lungworms between March 2022 and May 2023, and they were 18 males and 18 females (7 spayed or castrated), ageing from 10 weeks to 15 years, and all were allowed to free-roam or were living outdoor.

Each cat was enrolled upon informed consent signed by the owner. Cats with either a monospecific infection by *A. abstrusus* or *T. brevior* or infected by both parasites were divided in Group 1 (G1) and Group 2 (G2). The ratio between groups was 1:1, with random allocation based on order of presentation. Cats from multiple households and meeting the inclusion criteria were assigned to the same group. Personnel involved with parasitological and clinical evaluations and assessment of safety were unaware as to treatment.

Thirty-six cats completed the study, 18 in each of the two study Groups. In each G1 and G2, 6, 6 and 6 cats infected by *A. abstrusus*, *T. brevior* and with mixed infection, respectively, were included.

Animals in G1 were treated twice at Study Days (SDs) 0 and 28±2 with a formulation containing eprinomectin, esafoxolaner and praziquantel (NexGard® Combo, Boehringer Ingelheim Animal Health) and clinically evaluated for up to 32 weeks (Table 1). Cats in G2 were initially left untreated receiving mineral oil as a placebo and served in the study first as a negative control group compared to G1 over a period of 8 weeks. Thereafter, cats of G2 were treated with NexGard® Combo twice at a 4-week interval on SDs 56±2 and 84±2, for an efficacy evaluation per comparison with baseline data until SD 112±2 (Table 1).

Study cats were treated according to label indications for aelurostrongylosis and troglostrongylosis.

Specifically, all cats were subjected to quali-quantitative Baermann and PCR on Baermann sediment, clinical examination, two orthogonal thoracic radiographs and blood sampling for complete blood count and serum chemistry according to the study schedule reported in Table 1.

2.2. Baermann technique

The qualitative Baermann test was performed to evaluate positivity/negativity of cats by the examination of 5–10 g of fecal material. First stage larvae (L1) of *A. abstrusus* and *T. brevior* were identified based on key morphological and morphometric features (reviewed in Morelli et al., 2021).

The quantitative Baermann test was performed for positive cats, using 4 g of faeces for each sample. As previously described, based on the test sensitivity, estimated to be 15 larvae per gram of faeces (LPG), the parasitic burden was calculated by multiplying X15 the number of larvae counted in the microscopic field (Traversa et al., 2009a, b).

Between SD −7 and 0, all Baermann sediments were subjected to a

Table 2
Anthelmintic efficacy against *Aelurostrongylus abstrusus* and/or *Troglostrongylus brevior* in single and mixed infections.

		Group 1 Mean LPG	Standard Deviation (Min-Max)		Group 2 Mean LPG	Standard Deviation (Min-Max)	% Efficacy in G1, G2 as control	% Efficacy in G2 to G2 baseline
Aelurostrongylus abstrusus in single infections								
SD 0	T1	115.00	103.34 (45–315)		67.50	31.10 (45–120)		
SD 28	T2	7.50	12.55 (0–30)		77.50	58.03 (30–180)	90.32	
SD 56		0.00	0.00	T1	70.00	56.66 (30–180)	100.00	
SD 84				T2	2.50	6.12 (0–15)		96.43
SD 112					0.00	0.00		100.00
Troglostrongylus brevior in single infections								
SD 0	T1	82.50	49.07 (15–150)		82.50	55.92 (15–180)		
SD 28	T2	2.50	6.12 (0–15)		70.00	45.17 (15–120)	96.43	
SD 56		0.00	0.00	T1	80.0	42.07 (30–135)	100.00	
SD 84				T2	0.00	0.00		100.00
SD 112					0.00	0.00		100.00
Aelurostrongylus abstrusus in mixed infections								
SD 0	T1	27.50	11.29 (15–45)		110.00	107.19 (15–300)		
SD 28	T2	0.00	0.00		85.00	95.66 (15–270)	100.00	
SD 56		0.00	0.00	T1	105.00	116.96 (15–330)	100.00	
SD 84				T2	0.00	0.00		100.00
SD 112					0.00	0.00		100.00
Troglostrongylus brevior in mixed infections								
SD 0	T1	37.50	35.18 (15–105)		57.50	43.90 (15–120)		
SD 28	T2	5.00	12.25 (0–30)		75.50	46.48 (30–150)	93.33	
SD 56		0.00	0.00	T1	50.00	29.50 (15–90)	100.00	
SD 84				T2	0.00	0.00		100.00
SD 112					0.00	0.00		100.00

Group 1 (G1): 18 cats treated with NexGard® Combo at Study Days (SD) 0 and 28 ±2; Group 2 (G2): 18 cats treated with NexGard® Combo at Study Days 56 ±2 and 84 ±2. T1: first treatment; T2: second treatment. LPG: Larvae Per Gram of faeces.

diagnostic nested PCR specific for *A. abstrusus* or *T. brevior* to confirm the identification at the species level (Di Cesare et al., 2014).

2.3. Clinical examinations

At each timepoint (Table 1) the presence/absence of clinical and radiographic signs were recorded and clinical (CS) and radiographic (RS) scores were defined as previously described (Crisi et al., 2020). In detail, 0–2 points were assigned for each respiratory and non-respiratory clinical sign, while 0–3 points were assigned for each radiographic alteration. A total CS and RS was defined for individual cats at each visit by adding the scores of each alteration detected. A median score of cats belonging to G1 and G2 was also defined.

Blood samples were obtained according to the schedule in Table 1. Complete blood cell count (CBC) and serum chemistry values were evaluated at each SD for varying number of cats (detailed information is reported in supplemental material).

2.4. Efficacy criteria and statistical analysis

The assessment of treatment response was based on anthelmintic efficacy and clinical examinations, as primary and secondary efficacy criteria respectively.

The anthelmintic efficacy was evaluated within G1 based on the reduction of the L1 count (LPG) from SD −7/0 (baseline) to post-treatment evaluations at SD 28± 2 (I post-baseline) and SD 56±2 (II post-baseline) or from the comparison of G2 LPG at SD 28 and SD 56 using the following formula:

% reduction = (Mean LPG at baseline−Mean LPG at post-baseline) / Mean LPG at baseline × 100 % reduction = (Mean LPG G1 −Mean LPG G2 at SD28/56) / Mean LPG at SD28/56 × 100

The anthelmintic efficacy within G2 was evaluated based on the reduction of the L1 count (LPG) from SD 56 (baseline) to post-treatment evaluations at SD 84 (I post-baseline) and SD 112 (II post-baseline) (Table 1).

Mean LPG values were calculated as arithmetic mean.

All LPG counts were analyzed by Wilcoxon non-parametric sum rank test (= Mann-Withney test) and all testing were two-sided at the significance level α=0.05.

Statistical analyses were performed using Excel software and BiostaTGV® website (https://biostatgv.sentiweb.fr/), and the R statistical program.

Clinical response was assessed through the comparison and reduction of the CS and RS from the baseline to each follow-up. CS and RS were compared to baseline within G1 and between G1 and G2. The presence/absence of respiratory clinical signs was evaluated at baseline and all post-treatment visits. Post-treatment presence/absence of clinical signs was compared to the baseline within G1 and between G1 and G2. The pre- and post-treatment occurrence of alterations of hematology and serum biochemistry was evaluated compared to the baseline, within G1, within G2 and between G1 and G2. To obtain additional efficacy data the same evaluations (CS, RS and laboratory results) were performed for G2 after the rescue treatment, according to the schedule reported in Table 1.

Table 3
p value based on the distribution of LPG at each timepoint in cats of Group 1 (G1) and Group 2 (G2) with single and mixed infection by *Aelurostrongylus abstrusus* (*A.a.*) and/or *Troglostrongylus brevior* (*T.b.*) treated with NexGard® Combo. Significant difference is considered with p-value < 0.05.

	SD	*A.a.* single infections	*T.b.* single infections	*A.a.* in mixed infections	*T.b.* in mixed infections
P value					
G1/G2	−7/0	0.4541	1	0.1181	0.3227
G1/G2	28	**0.0067**	**0.0046**	**0.0027**	**0.0057**
G1/G2	56	**0.0027**	**0.0027**	**0.0027**	**0.0027**
G2/G2	28/56	0.8698	0.6285	0.6857	0.3682
G2/G2	56/84	**0.0036**	**0.0027**	**0.0027**	**0.0027**
G2/G2	56/112	**0.0027**	**0.0027**	**0.0027**	**0.0027**

G1: 18 cats treated with NexGard® Combo at Study Days 0 and 28 ±2; G2: 18 cats treated with NexGard® Combo at Study Days (SD) 56 ±2 and 84±2.
*significant values evidenced by bold type.

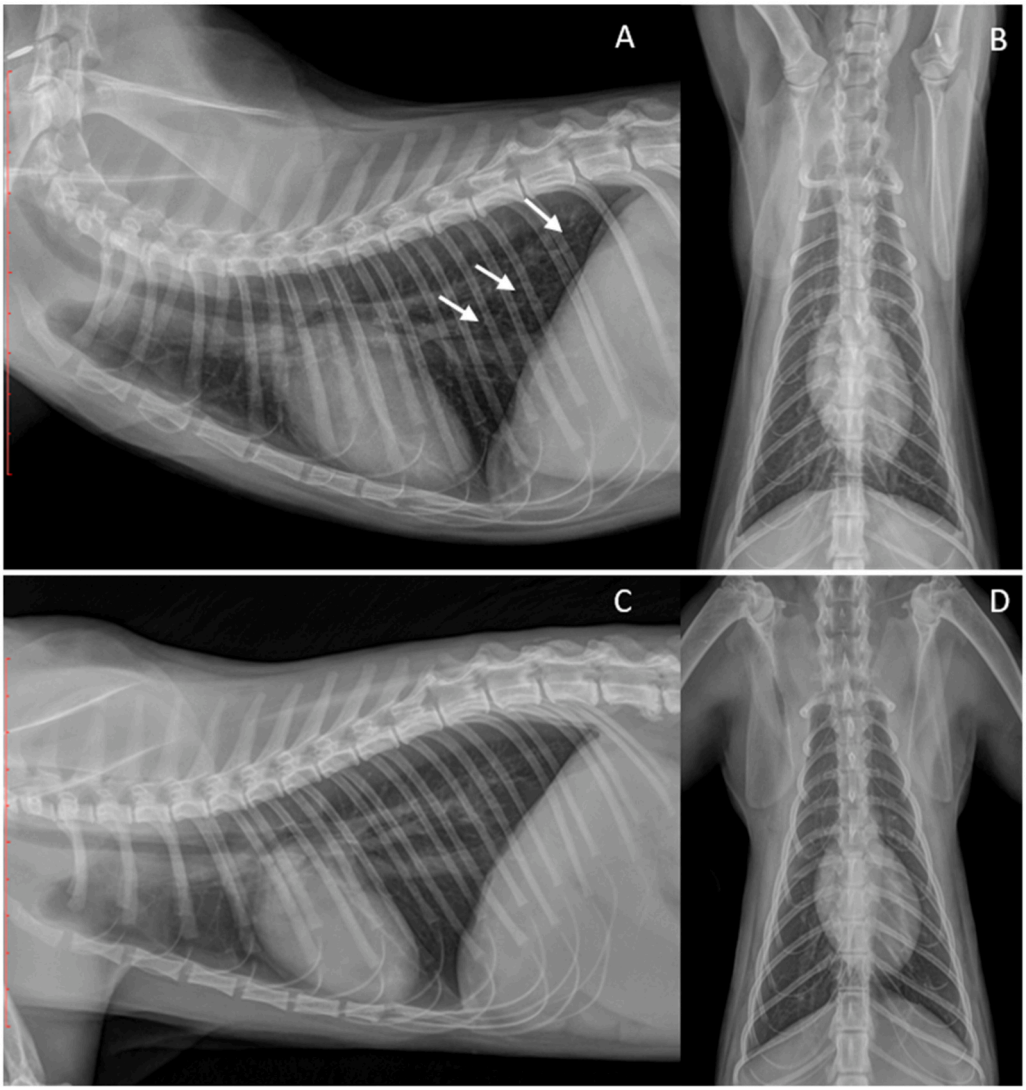

Fig. 1. Right lateral and ventrodorsal views of the thorax of a 2-year-old male cat with *Troglostrongylus brevior* infection, at SD −7/0 (A, B) and at SD 56±2 (C, D). Before treatment a bronchial pattern, with severe peribronchial cuffing in the caudo-dorsal lobes (white arrows) is visible on right lateral projection (A). At SD 56±2 (C, D), the cat showed the resolution of radiographic signs.

3. Results

None of the cats showed adverse reactions after the administration of NexGard® Combo or received concomitant treatments.

At baseline (SD 0) no significant differences between clinical, laboratory and radiographic findings were observed between G1 and G2.

Overall, 16 cats in G1 completed the study on SD 56±2, with 2 cats that still had radiographic lesions were further evaluated until SD 84±2 and 112±2, respectively. Eighteen cats in G2 completed the study until SD 112±2.

3.1. Anthelmintic efficacy

Four and 5 G1 cats with monospecific infections by *A. abstrusus* or

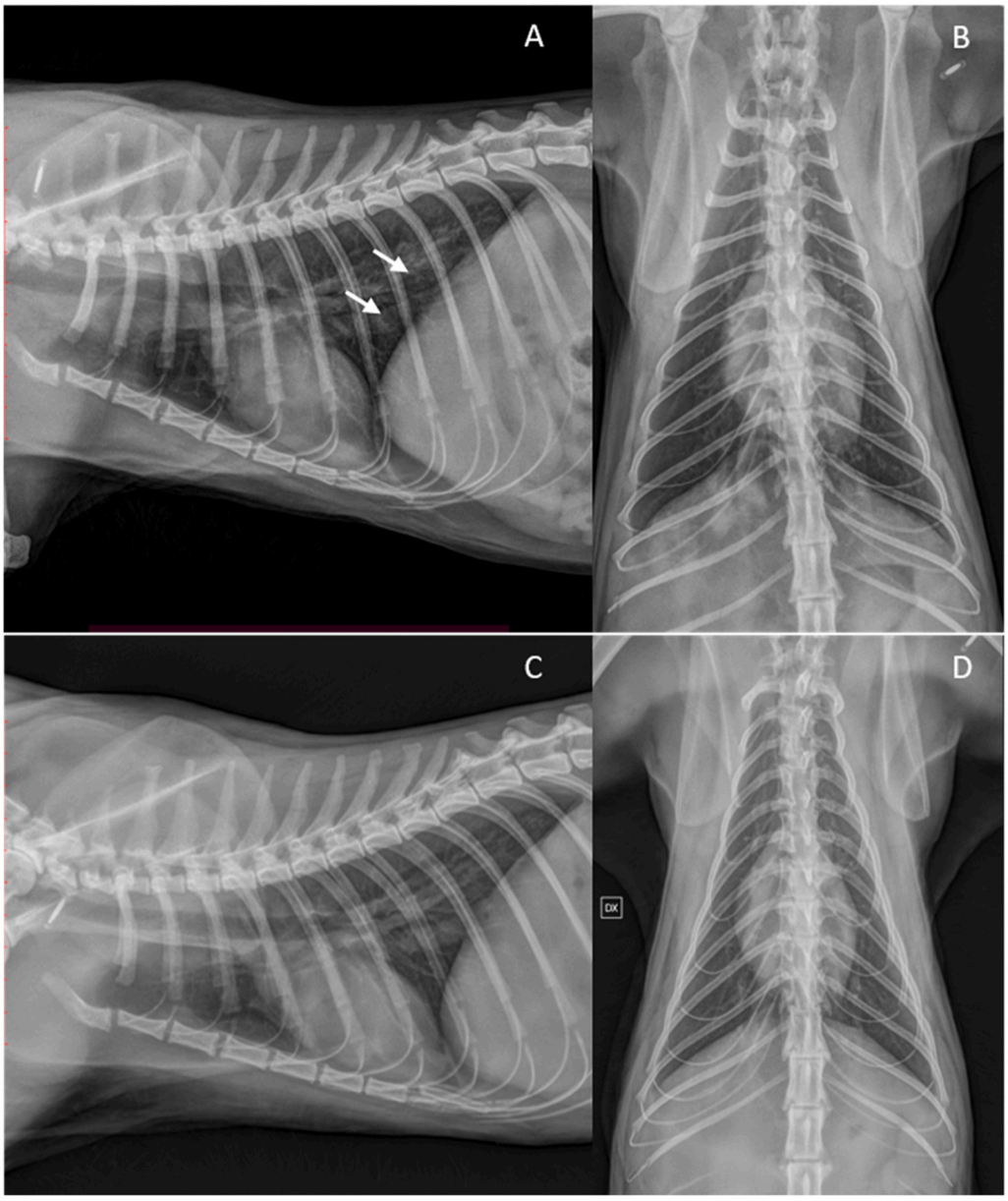

Fig. 2. Right lateral and ventrodorsal views of the thorax of a 7-year-old cat with *Aelurostrongylus abstrusus* infection, at SD −7/0 (A, B) and at SD 112±2 (C, D). Before treatment a bronchial pattern, with severe peribronchial cuffing in the caudo-dorsal lobes (white arrows) is visible on right lateral projection (A). At SD 112±2 (C, D), the cat showed the resolution of radiographic signs.

T. brevior respectively scored negative for larvae at SD 28±2. At the same SD, 5 cats of the same group infected by both lungworms were then negative. All G1 cats were negative at the Baermann's test at SD 56±2.

Untreated cats in G2 were persistently infected until SD 56±2. All of them became negative for larvae at both SDs 84±2 and 112±2, with the exception of a cat infected with *A. abstrusus* which scored positive at SD 84±2 and was negative at SD 112±2.

The mean LPG counts of cats belonging to G1 and G2 at each time-point are listed in Table 2.

A complete disappearance of L1s was observed after two treatments in all cats (Table 2) and the results of the non-parametric Wilcoxon sum rank test showed that the distribution of LPG was significantly different between G1 and G2 after one and two treatments (Table 3). Specifically, on SD 28±2 the reduction of LPG counts in G1 cats compared to G2 was 90.32 % ($p = 0.0067$) and 96.43 % ($p = 0.0046$) for cats infected by *A. abstrusus* or *T. brevior*, respectively. In mixed infections the reduction of LPG count on SD 28 ±2 was 100 % ($p = 0.0027$) for *A. abstrusus* and 93.33 % ($p = 0.0057$) for *T. brevior*. At SD 56±2 the efficacy was 100 % ($p = 0.0027$) for all G1 cats.

In G2 cats, the reduction of LPG counts on SD 84±2 compared to the baseline (i.e. SD 56 ±2) was 96.43 % ($p = 0.0036$) for cats infected only by *A. abstrusus* and 100 % ($p = 0.0027$) for those infected only by *T. brevior* or with mixed infection. On SD 112±2 all G2 cats were negative for larvae (efficacy 100 %, $p = 0.0027$).

3.2. Clinical Efficacy

On SD −7/0, all 36 cats showed clinical signs and/or radiographic alterations (Figs. 1, 2 and 3, Tables 4 and 5). Sixteen cats in G1 showed both clinical signs and radiographic alterations, while 1 cat showed only clinical signs and another had only radiographic alterations. On SD 28 ±2, clinical and radiographic signs persisted in 10 G1 cats, while 7 cats exhibited only radiographic signs and 1 cat was healthy. On SD 56±2, 15 G1 cats were healthy, while one cat infected with *T. brevior* displayed

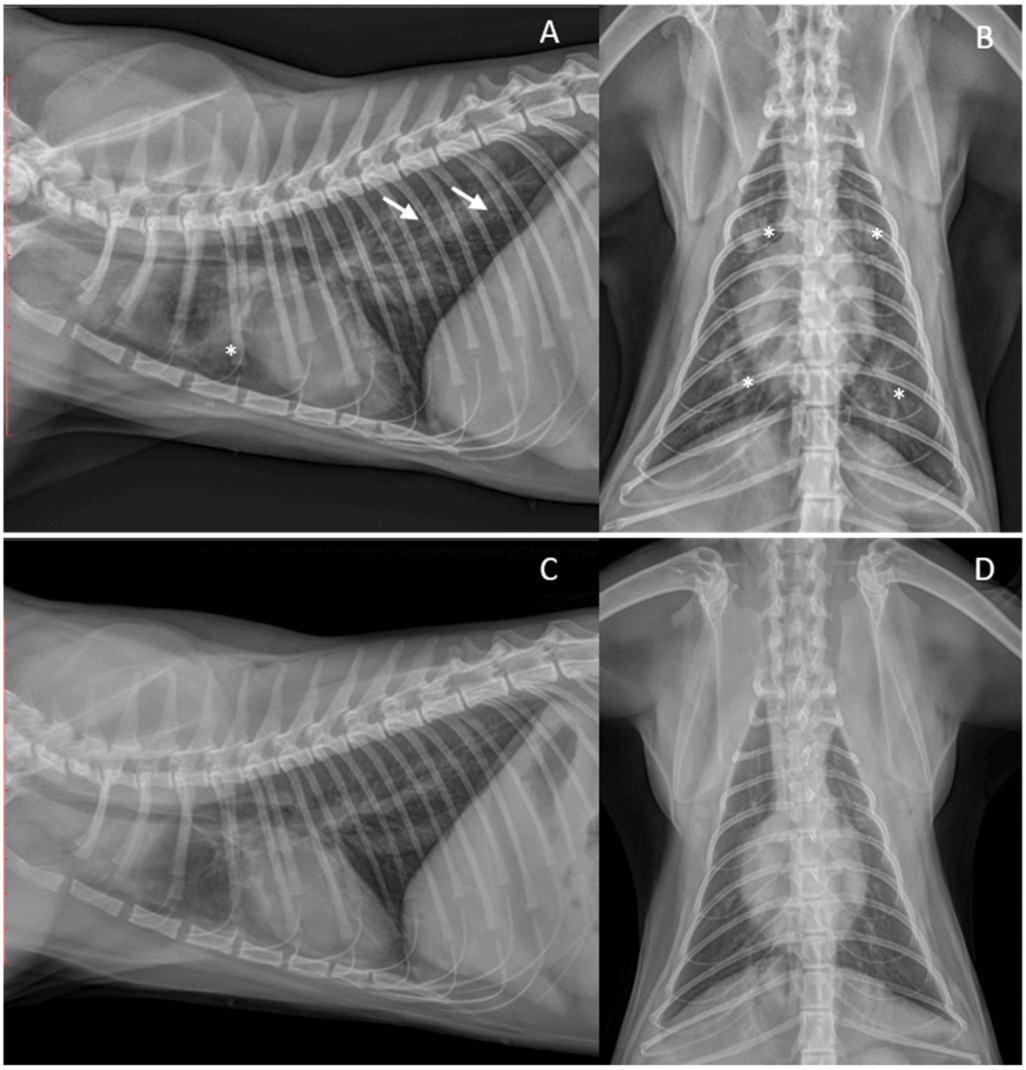

Fig. 3. Right lateral and ventrodorsal views of the thorax of a young adult cat with *Troglostrongylus brevior* and *Aelurostrongylus abstrusus* co-infection, at SD −7/0 (A, B) and at SD 56±2 (C, D). Before treatment a bronchial pattern with moderate peribronchial cuffing (white arrows) (A) and a mixed interstitial pattern (white asterisks) are present (A and B). At SD 56±2 (C, D), the cat showed the resolution of radiographic signs.

clinical signs despite radiographic alterations no longer being detectable. At the same SD, resolution of clinical signs occurred in another cat infected with *T. brevior* but radiographic alterations were still visible. In 1 cat infected with *A. abstrusus* both clinical and radiographic signs persisted. According to the study protocol (i.e. cats that showed radiographic alterations had to be further examined), the latter two cats underwent further examinations until SD 84±2 and 112±2, respectively, with absence of clinical or radiographic signs in one cat at SD 84 ±2 and in the other cat at 112±2.

Among cats in G2, 16 showed clinical and/or radiographic signs at SD 28±2; at SD 56±2, 15 exhibited respiratory clinical signs and radiographic alterations and 3 cats showed only clinical signs. On SD 84 ±2, clinical and radiographic signs were still evident in 7 cats, 5 cats exhibited only respiratory clinical signs, 4 only radiographic alterations and 2 cats were healthy. At SD 112±2 clinical and radiographic abnormalities disappeared in all G2-cats except for one cat originally infected by *T. brevior* before treatment that still showed clinical signs.

Detailed information about the number of cats showing clinical and radiographic signs and mean CS and RS is reported in Tables 4 and 5.

Overall, CS and RS were reduced after the first treatment and significant clinical and complete radiographic recovery (Figs. 1, 2 and 3) was observed after two monthly treatments in all cats regardless of monospecific or mixed infections (Table 6). Blood count and serum chemistry mean values are reported in the supplementary material.

At SD −7/0 the most common hematological abnormalities were eosinophilic leukocytosis (in 13 cats) and thrombocytopenia (in 12 cats). Three cats had neutrophilia before treatment. Regarding biochemical analysis, 7 cats showed mild increased aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) levels. After two monthly treatments, 4 cats had eosinophilia, 8 thrombocytopenia and 7 neutrophilia. Nine cats had high value of ALT, AST and/or ALP. After treatments neutrophils in G1 cats infected with *A. abstrusus* were significantly increased ($p = 0.037$) (Table 7).

4. Discussions

Eprinomectin is a macrocyclic lactone with a broad-spectrum activity against various internal nematodes, including *A. abstrusus* and *T. brevior* as shown one decade ago (Knaus et al., 2014; Giannelli et al., 2015). In 2021, a new formulation containing eprinomectin, praziquantel and esafoxolaner has been marketed in the EU, and it is licensed for treating the infections caused by *A. abstrusus* and *T. brevior*. The results of this study confirm the high efficacy and safety of eprinomectin in interrupting lungworm larval shedding in naturally infected cats (Knaus

Table 4
Number of cats showing clinical signs and mean clinical score (CS) observed in 36 cats infected with *Aelurostrongylus abstrusus* and/or *Troglostrongylus brevior* at each timepoint.

SD	Group	S n/tot	W n/tot	A n/tot	C n/tot	MM n/tot	D n/tot	RR n/tot	RM n/tot	AU n/tot	Tot	Mean CS
Aelurostrongylus abstrusus												
−7/−0*	G1	3	1	0	3	0	1	3	2	5	6	2.6
	G2	0	2	0	4	0	1	2	0	3	5	1.8
28±2*	G1	2	0	0	1	0	1	0	0	2	3	0.6
	G2	0	2	0	4	0	1	1	0	3	5	1.6
56±2**	G1	0	0	0	0	0	0	0	0	1	1	0.1
	G2	0	2	0	5	0	1	1	0	3	6	2.0
84±2**	G1#	0	1	0	0	0	0	0	0	1	1	-
	G2	0	1	0	4	0	1	0	0	0	5	1.0
112±2	G1#	0	0	0	0	0	0	0	0	0	0	-
	G2	0	0	0	0	0	0	0	0	0	0	0
Troglostrongylus brevior												
−7/−0*	G1	1	1	1	4	0	1	4	4	3	5	4.3
	G2	1	1	0	3	0	1	4	3	4	5	2.6
28±2*	G1	0	0	0	2	0	0	3	2	3	3	1.8
	G2	1	0	0	4	0	1	1	1	5	5	2
56±2**	G1	0	0	0	0	1	0	1	0	0	1	0.3
	G2	1	1	0	5	0	1	3	2	5	6	2.8
84±2**	G1##	0	0	0	0	0	0	0	0	0	0	-
	G2	0	0	0	1	0	0	1	0	3	3	1.0
112±2	G1	-	-	-	-	-	-	-	-	-	-	-
	G2	0	0	0	0	0	0	1	0	0	1	0.1
Aelurostrongylus abstrusus + *Troglostrongylus brevior*												
−7/−0*	G1	1	2	1	1	0	1	6	6	4	6	4.1
	G2	2	1	1	2	1	1	3	3	6	6	3.1
28±2*	G1	0	0	0	2	0	0	1	1	2	4	1.1
	G2	1	1	0	2	1	1	3	3	6	6	3.0
56±2**	G1	0	0	0	0	0	0	0	0	0	0	0
	G2	1	0	0	3	0	1	3	3	6	6	3.1
84±2**	G1	-	-	-	-	-	-	-	-	-	-	-
	G2	0	0	0	2	1	0	1	0	4	4	1.3
112±2	G1	-	-	-	-	-	-	-	-	-	-	-
	G2	0	0	0	0	0	0	0	0	0	0	0

Group 1 (G1): 18 cats treated with NexGard® Combo at Study Days (SD) 0 and 28 ±2; Group 2 (G2): 18 cats treated with NexGard® Combo at Study Days 56 ±2 and 84 ±2. S: Sneezing; W: Wheezing; A: Decreased activity; MM: Pale mucous membranes, D: Ocular-nasal discharge, RR: respiratory rate; RM: respiratory movements; AU: Auscultation. Data on Lymphatic, Circulatory, Nervous, Genito-Urinary systems are not shown as no alterations were found. * Treatment of cats from G1; ** Treatment of cats from G2. # 1 cat from G1 showing radiographic alterations On SD 56±2 and subjected to a further clinical and radiographic evaluation on SD 84±2 and 112±2; ## 1 cat from G1 showing radiographic alterations On SD 56±2 and subjected to a further clinical and radiographic evaluation on SD 84.

et al., 2014; Giannelli et al., 2015). Moreover, in the present study two administrations 28 days apart of NexGard® Combo were also highly effective in terms of clinical recovery for cats with natural aelurostrongylosis and/or troglostrongylosis. In fact, almost all cats in G1 completely recovered from clinical signs and radiographic alterations within 8 weeks after the first administration. The fact that 2 cats of G1 with monospecific infections by *A. abstrusus* and *T. brevior* required 16 and 12 weeks respectively for a complete radiographic recovery could be hypothetically due to individual factors, e.g. immune system efficiency, persistence of adult nematodes in absence of larval excretion, extent of pulmonary damages. In fact, the cat with monospecific troglostrongylosis had a higher baseline clinical score than most cats included in the study. Moreover, both cats showed a high pre-treatment radiographic score with severe alveolar and mild reticular interstitial patterns, which may also have influenced the recovery duration. Previous data obtained in a case series study (Crisi et al., 2017) suggest that cats with more severe clinical pictures may require a longer period to heal from clinical and radiographic alterations after anthelminthic treatment, which corroborates these results. On the other hand, the presence of cats with severe clinical and radiographic pictures that underwent a faster resolution of alterations suggest that individual susceptibility to treatment and recovery capacities may impact the time required for complete recovery (Crisi et al., 2017; present data). The duration recorded here between the first treatment and the remission of clinical and radiographic signs in most cats infected by *A. abstrusus* only (i.e. 8 weeks) fits with the information gathered in a previous study (i.e. 10 weeks) evaluating the clinical efficacy of spot-on emodepside (Crisi et al., 2020). Nevertheless, further thorough and accurate comparison with the latter clinical study is not possible, due to a different study design, e.g. different treatment administration regimens of the anthelminthic and evaluation timepoints in terms of SDs.

Data on clinical efficacy in cats infected by *T. brevior* under natural conditions were previously obtained in field studies evaluating the efficacy of different anthelmintic actives, in single case reports and from case series. The use of spot on moxidectin 1 % has shown efficacy in previous reports but further comparison is not possible as data originated from a case series with no randomization, blinding and with a limited number of animals (Crisi et al., 2017) or from a study in cats with subclinical infections (Diakou et al., 2019). A pilot trial showed the efficacy of emodepside in clinical recovery of naturally infected cats, but in this other study, pulmonary radiographic alterations or clinic-pathological alteration were not investigated (Traversa et al., 2019).

Radiographic alterations described for aelurostrongylosis and troglostrongylosis are usually non-specific and mixed patterns are often observed (Crisi et al., 2017; Febo et al., 2019; Morelli et al., 2021). The more frequent thoracic radiograph alterations observed here in cats with aelurostrongylosis (i.e. bronchial and interstitial reticular patterns), and with troglostrongylosis (i.e. bronchial and interstitial reticular pattern) are in line with previous data (Brianti et al., 2012; Crisi et al., 2017; Febo et al., 2019; Morelli et al., 2021). However, the absence of nodular interstitial pattern in cats in the present trial is in contrast with the observations in previous studies (Brianti et al., 2012; Crisi et al., 2017; Febo et al., 2019). These results further underline the non-specificity of radiographic findings in cats with lungworms. Even though thoracic radiographic abnormalities can raise suspicion of infection with

Table 5
Number of cats showing radiographic alterations and mean radiographic score (RS) observed in 36 cats infected with *Aelurostrongylus abstrusus* and/or *Troglostrongylus brevior* at each timepoint.

SD	Group	BP n/tot	AP n/tot	RP n/tot	Total	Mean RS
Aelurostrongylus abstrusus						
−7/−0*	G1	6	1	2	6	2.5
	G2	4	0	3	5	2.1
28±2*	G1	6	1	1	6	2.1
	G2	3	1	3	4	2.3
56±2**	G1	1	0	1	1	0.6
	G2	3	1	4	5	2.0
84±2**	G1#	1	-	0	1	-
	G2	3	0	1	3	1.0
112±2	G1#	0	0	0	0	-
	G2	0	0	0	0	0
Troglostrongylus brevior						
−7/−0*	G1	5	0	2/6	5	2.3
	G2	4	0	4/6	6	1.8
28±2*	G1	5	0	1	5	1.5
	G2	4	0	3	5	1.6
56±2**	G1	1	0	0	1	0.1
	G2	4	0	2	4	1.8
84±2**	G1##	0	0	0	0	-
	G2	4	0	1	4	1.8
112±2	G1	-	-	-	-	-
	G2	0	0	0	0	0
Aelurostrongylus abstrusus + *Troglostrongylus brevior*						
−7/−0*	G1	6	0	0	6	1.3
	G2	6	1	3	6	3.0
28±2*	G1	6	0	0	6	1.8
	G2	6	1	3	6	3.0
56±2**	G1	0	0	0	0	0
	G2	6	1	3	6	3.1
84±2**	G1#	-	-	-	-	-
	G2	4	0	2	4	1.6
112±2	G1#	-	-	-	-	-
	G2	0	-	0	0	0

Group 1 (G1): 18 cats treated with NexGard® Combo at Study Days (SD) 0 and 28 ±2; Group 2 (G2): 18 cats treated with NexGard® Combo at Study Days 56 ±2 and 84±2. BP: bronchial pattern; AP: alveolar pattern; RP: reticular interstitial pattern; # cats from G1 showing radiographic alterations On SD 56 and subjected to a further clinical and radiographic evaluation on SD 84±2 and 112 ±2; * Treatment of cats from G1; ** Treatment of cats from G2. # 1 cat from G1 showing radiographic alterations On SD 56 and subjected to a further clinical and radiographic evaluation on SD 84±2 and 112±2; ## 1 cat from G1 showing radiographic alterations on SD 56±2 and subjected to a further clinical and radiographic evaluation on SD 84±2.

respiratory nematodes, they do not differentiate with those present in other pulmonary diseases. It should also be considered that radiographic abnormalities may vary depending on the pathological changes caused by parasite burden and duration of infection, and that their severity and distribution is influenced by immune response of the host, and the presence of concurrent respiratory diseases (rev. in Morelli et al., 2021). Also, there is only partial correlation between occurrence/severity of clinical signs and radiographic changes, as many infected cats showed radiographic changes without evident clinical signs (Crisi et al., 2017; Febo et al., 2019). Additionally, the usual behavior of domestic cats living mainly indoor may render difficult for the owner to observe impairment of pulmonary capacity with subclinical or mild signs. Though radiographic imaging does not provide an etiological diagnosis of feline lungworm disease, it represents an important tool in monitoring the recovery process and follow-up after treatment.

As an example, aelurostrongylosis is regarded as one of the main causes of intra-anesthetic deaths in cats (Gerdin et al., 2011). Thus, a careful clinical and radiographic evaluation prior to anesthetic procedures in cats with recent history of lungworms is herein recommended. If the cat is collaborative, neither sedation nor anesthesia is needed during radiological examination. However, some cats with fractious temperaments or excited behavior may benefit from sedation to reduce anxiety and stress during diagnostic procedures as imaging (Simon and Steagall, 2020). In such cases, clinicians should consider the risk that sedation (or anesthesia) may pose for a cat infected with lungworms. This risk can be

Table 6
p value based on the distribution of Clinical and Radiographic Scores at each timepoint in cats of Group 1 (G1) and Group 2 (G2) with mono and mixed infection by *Aelurostrongylus abstrusus* (*A.a.*) and *Troglostrongylus brevior* (*T.b.*) treated with NexGard® Combo. Significant difference* is considered with p-value < 0.05.

		Clinical score (*p* value)			Radiographic score (*p* value)		
	SD	*A.a.*	*T.b.*	*A.a./T.b.*	*A.a.*	*T.b.*	*A.a./T.b.*
G1/G2	−7/0	0.2840	0.6279	0.3306	0.6248	0.8018	0.1422
G1/G2	28	0.1119	0.8693	0.0784	0.8698	0.8683	0.2749
G1/G2	56	**0.0056**	**0.0111**	**0.0026**	0.0698	0.0602	**0.0027**
G2/G2	28/56	0.6734	0.4408	0.8058	0.9344	0.9346	0.9344
G2/G2	56/84	0.0698	0.0841	0.1012	0.3611	1	0.1938
G2/G2	84/112	**0.0081**	0.2144	**0.028**	0.0740	**0.0284**	**0.0284**
G2/G2	56/112	**0.0026**	**0.0044**	**0.0027**	**0.009**	**0.0284**	**0.0027**

Group 1 (G1): 18 cats treated with NexGard® Combo at Study Days (SD) 0 and 28 ±2; Group 2 (G2): 18 cats treated with NexGard® Combo at Study Days 56 ±2 and 84±2.
*significant values evidenced by bold type

Table 7
p value based on the distribution of hematology and clinical chemistry mean score in cats of Group 1 (G1) and Group 2 (G2) treated with NexGard® Combo. Significant difference* is considered with p-value < 0.05.

	p-value		
Haematobiochemical parameters#	**G1/G2 SD −7/0**	**G1/G2 SD 56**	**G2/G2 Day 56/112**
Aelurostrongylus abstrusus			
Erythrocytes mm^3	0.970	0.528	
Platelets mm^3	0.762	0.254	
MPV	0.684	0.097	
Eosinophils %	0.326	0.759	
Neutrophilis mm^3	0.975	**0.037**	0.343
Alkaline phosphatase	0.617	0.610	
Troglostrongylus brevior			
WBC	0.760	0.193	
MCV	0.256	0.087	
Monocytes	0.322	0.994	
Neutrophilis	0.369	0.480	
Aelurostrongylus abstrusus* and *Troglostrongylus brevior			
Erythrocytes mm^3	0.327	0.597	
Platelets mm^3	0.546	0.407	
MCV	0.904	0.624	
MPV	0.620	0.734	
Lymphocytes %	0.963	0.715	
Monocytes %	0.945	0.550	
Neutrophils %	0.367	0.512	
Eosinophils %	0.0594	0.239	
Lymphocytes mm^3	0.225	0.114	
Monocytes mm^3	0.355	0.411	
Eosinophilis mm^3	0.143	0.936	
Alanine transaminase	0.739	0.0972	
Alkaline phosphatase	0.067	0.298	
total glucose mg/dl	0.413	0.461	

#The White Blood Cell differential count was determined for 24 cats.
Group 1 (G1): 18 cats treated with NexGard® Combo at Study Days (SD) 0 and 28 ±2; Group 2 (G2): 18 cats treated with NexGard® Combo at Study Days 56 ±2 and 84±2.
*significant values evidenced by bold type

minimized by an appropriate stabilization of the patient, a careful monitoring of the cat and supportive care plan (Simon and Steagall, 2020).

Neutrophilia may be present during aelurostrongylosis and troglostrongylosis (Crisi et al., 2017). The increased eosinophil count, one of the most frequent hematological alterations found here, in both single or mixed infections, fits with existing literature (Foster et al., 2004; Diakou et al., 2014; Schnyder et al., 2014; Crisi et al., 2015; 2017, 2018). Though not pathognomonic of lungworm infection, eosinophilia is frequent in cats with parasitic disease and, when detected, flotation and Baermann test should be included in the diagnostic strategy. Thrombocytopenia has already been described in cats infected with *A. abstrusus* and *T. brevior* (Umur et al., 2020; Napoli et al., 2023) and it was also recorded in cats in the present study. It was previously hypothesized that platelet consumption in cats infected by lungworms may occur as a consequence of subclinical disseminated intravascular coagulation caused by arterial damages or of immune-mediated thrombocytopenia (Napoli et al., 2023). Potential hepatic injury has been described in cats with lower airway diseases, including aelurostrongylosis (Foster et al., 2004; Crisi et al., 2017). Similar alterations have been here recorded and described for the first time in cats with troglostrongylosis. Additionally, a lack of normalization of liver enzymes values after treatment was here found in accordance to a previous study (Crisi et al., 2020). These biochemical alterations in cats with lungworms are non-specific and they could be due to hypoxia and/or to a generalized inflammation status (Crisi et al., 2017) and do not necessarily indicate a permanent liver damage. Therefore, the factual clinical significance of these pathological findings is yet to be clarified and should be further investigated.

The efficacy evaluations relying on copromicroscopic examinations may be potentially impaired by the possibility that parasiticides stop larval excretion only temporarily. However, experimental studies have already demonstrated that most cats experimentally infected by *A. abstrusus* or *T. brevior* and which did not shed parasitic L1 after treatment were negative for worms or had dead worms at necropsy (Böhm et al., 2015; Heuer et al., 2020; Knaus et al., 2020). Other laboratory studies have shown the potential of diagnostic imaging either in the pathological evaluation of aelurostrongylosis or in post-treatment follow up of artificially infected animals (Dennler et al., 2013; Raue et al., 2021). Therefore, the clinical and radiological examinations here performed strongly support the successful elimination of adult parasites in study cats with natural infections.

The present data and previous clinical information (Crisi et al., 2020), suggest that, in most cases, feline lungworm infections undergo a similar post-treatment outcome, that encompasses three main phases, i.e. (i) an initial improvement of clinical signs followed by (ii) resolution of radiographic alterations and, thereafter, (iii) varying degrees of normalization of laboratory abnormalities (e.g. eosinophilic leukocytosis).

In conclusion, the administration of a spot-on formulation containing eprinomectin was shown to be highly effective under a clinical point of view to treat aelurostrongylosis and troglostrongylosis in both single and mixed infections. The use of effective parasiticides should be supported by a correct management of cats relying on a multimodal approach. Once a positive diagnosis is obtained, cats should be subjected to a complete physical examination, including thorax radiography and hematobiochemical analysis.

The follow-up should be performed monthly, and it should include the aforementioned analyses, along with the Baermann test. A fortnightly post-treatment monitoring of cats with aelurostrongylosis has been proposed (Crisi et al., 2020). Probably a monthly check-up with less frequent clinical examinations is a more suitable and reliable alternative to increase owner compliance and limit the level of stress for cats, which is a key variable in the veterinary management of feline patients (Quimby et al., 2011).

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CRediT authorship contribution statement

Raffaella Iorio: Methodology, Investigation. **Eric Tielemans:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Frederic Beugnet:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Fabrizia Veronesi:** Writing – review & editing, Methodology, Investigation, Data curation. **Paolo Emidio Crisi:** Methodology, Investigation, Formal analysis. **Camille Gamblin:** Formal analysis, Data curation. **Angela Di Cesare:** Writing – review & editing, Writing – original draft, Resources, Project administration, Formal analysis, Data curation, Conceptualization. **Donato Traversa:** Writing – review & editing, Writing – original draft, Validation, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Massimo Vignoli:** Methodology, Investigation, Data curation. **Giulia Rigamonti:** Methodology, Investigation. **Mariasole Colombo:** Methodology, Investigation. **Simone Morelli:** Methodology, Investigation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Donato Traversa and co-workers reports financial support was provided by Boehringer Ingelheim Animal Health. Eric Tielemans, Frederic Beugnet, Camille Gamblin reports a relationship with Boehringer Ingelheim Animal Health that includes: employment. Donato Traversa and Angela Di Cesare reports a relationship with Boehringer Ingelheim Animal Health that includes: consulting or advisory. This Study has been supported by Boehringer Ingelheim, of which ET, FB and CG are employees. DT and ADC have acted as consultants for Boehringer Ingelheim.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2024.110271.

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