OPEN ACCESS

PAPER

Controlled field study evaluating the clinical efficacy of a topical formulation containing emodepside and praziquantel in the treatment of natural cat aelurostrongylosis

Paolo Emidio Crisi ,[1] Angela Di Cesare,[1] Donato Traversa,[1] Massimo Vignoli,[1] Simone Morelli,[1] Morena Di Tommaso,[1] Francesca De Santis,[1] Fabrizio Pampurini,[2] Roland Schaper,[3] Andrea Boari[1]

Abstract

Background *Aelurostrongylus abstrusus* is the most important nematode affecting the respiratory tract of cats in terms of prevalence and clinical relevance. The aim of this randomised controlled field study was to confirm the efficacy of the spot-on containing emodepside/praziquantel (Profender, Bayer Animal Health) in the treatment of aelurostrongylosis.

Methods Seventeen cats with aelurostrongylosis and presenting with clinical and/or radiographic signs were included in the study. Eight cats received two biweekly doses of emodepside/praziquantel, while nine cats were allocated to a control group and received a rescue treatment at the end of the study. Clinical response was the primary outcome, while the secondary end point was the reduction of larval shedding in faeces.

Results Two weeks after the first application, the cats showed a significant, though partial, recovery of clinical signs with complete clinical and parasitological resolution. The resolution of inflammatory leucogram and a significant reduction of radiographic lesions were observed two weeks after the second treatment. Red blood cells and albumin values significantly increased after eight weeks from the second application, together with the complete regression of radiographic patterns.

Conclusion Two applications of this spot-on solution two weeks apart assured complete cessation of larval shedding and led to a complete clinical, clinicopathological and radiographic recovery.

Introduction

Aelurostrongylus abstrusus is the most important nematode affecting the respiratory system of domestic cats (*Felis silvestris catus*) in terms of geographical distribution and relevance in feline clinical practice. Cats become infected by ingesting intermediate (snails and slugs) or paratenic hosts such as rodents, birds, amphibians and reptiles.[1]

The parasite occurs worldwide and may infect all cats regardless of their habitat, lifestyle, sex and breed.[2] However, outdoor access and hunting behaviour are risk factors for the occurrence of *A abstrusus* infection.[3–6]

Clinical signs associated with feline aelurostrongylosis are due to the inflammatory reaction in the alveoli, bronchioles and arteries, elicited by egg production and hatching and migration of first-stage larvae (L1s) within the respiratory tract.[7 8] Clinical manifestations are variable: some animals are subclinically infected[9 10] or, more often, they present with prevailing respiratory and non-specific signs such as cough, dyspnoea, tachypnoea, sneezing, lethargy and weight loss.[3 4 11] Also, auscultation of the thorax usually reveals increased vesicular breath sounds, wheezing or crackles.[12] Severe infections, especially in kittens and immunocompromised cats, can cause more

Veterinary Record (2019) doi:10.1136/ vetrec-2019-105528

[1]Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy
[2]Bayer HealthCare, Milano, Italy
[3]Bayer Anim Hlth GmbH, Leverkusen, Germany

E-mail for correspondence: Dr Paolo Emidio Crisi; pecrisi@unite.it

Provenance and peer review Not commissioned; externally peer reviewed.

Received April 26, 2019
Revised August 1, 2019
Accepted October 5, 2019

serious clinical signs, including severe dyspnoea and occasionally death.[13 14]

Laboratory and radiographic abnormalities in aelurostrongylosis are non-specific and overlap those of other lower respiratory tract diseases.[9 15] The haematological abnormalities may include leucocytosis often associated with eosinophilia, mild normocytic and normochromic anaemia or, less frequently, basophilia, lymphocytosis and monocytosis. Biochemistry may show increased hepatic enzymes, alterations in clotting profile, such as increase of prothrombin time, and decreased activated partial thromboplastin time, thrombin time and fibrinogen.[12 16]

Thoracic radiography often reveals a pulmonary interstitial and bronchial infiltrative pattern, with alveolar and nodular patterns being less common. Thoracic radiography and findings depend on the stage of infection, infection dose and the stage of the disease, with radiographic alterations in apparently healthy but infected cats and a possible lack of radiographic lesions in the presence of overt clinical signs.[10 17]

To date, only few products are labelled to treat aelurostrongylosis, with differences among different countries. For example, some formulations containing fenbendazole (Panacur, MSD) are licensed in UK, and a spot-on formulation containing imidacloprid 10% w/v and moxidectin 1% w/v (Advocate, Bayer Animal Health) is licensed in Australia and recently received a positive opinion from the EU Committee for Medicinal Products for Veterinary Use of the European Medicines Agency (EMA CVMP) for the European label. In the EU two topical formulations containing emodepside 2.1% w/v and praziquantel 8.6% w/v (Profender, Bayer Animal Health) and fipronil 8.3% w/v, (S)-methoprene 10% w/v, eprinomectin 0.4% w/v and praziquantel 8.3% w/v (Broadline, Boehringer Ingelheim) are labelled for treating *A abstrusus* infection. Based on post-treatment larval counts, all of these products have shown an efficacy ranging around 90 per cent to 100 per cent in various experimental and field studies.[18–22] However, knowledge on clinical response to treatments needs to be improved, with a particular focus on time to resolution of clinical, laboratory and radiographic alterations.

Therefore, the aim of this study was to evaluate the efficacy of topical emodepside/praziquantel for treatment of naturally occurring feline aelurostrongylosis, and specifically to determine the treatment response for clinical signs, radiographic abnormalities, clinicopathological abnormalities and *A abstrusus* larval shedding.

Materials and methods

This study was a randomised controlled field trial. A pretreatment inclusion screening was performed on animals referred to the Veterinary Teaching Hospital of the University of Teramo (Italy) from April 2016 to June 2018. All animals were privately owned naturally infected cats for which each owner signed a consent form and accepted to participate in the study. Eligible animals were those that met, from day −15 to day −4, at least one of the following three criteria: *a*) a previous history of aelurostrongylosis; *b*) presence of respiratory signs and *c*) submitted to routine copromicroscopy and/or referred for other reasons. The cats underwent a complete clinical examination, with a thorough history, retroviral status (SNAP Feline Immunodeficiency Virus/Feline Leukaemia Virus Combo Test, IDEXX Laboratories), two orthogonal thoracic radiographs, Baermann test and PCRs from faeces and pharyngeal swabs. Clinical scores (CS) and a radiographic scores (RS) were assessed for each cat, as previously described.[10] For each clinical sign (ie, food intake, activity, cough, mucous membranes, rectal temperature, ocular and/or nasal discharge, respiratory rate, respiratory movements, auscultations) 0 to 2 points were assigned, and 0 to 3 points were assigned for each radiographic alteration (ie, bronchial, alveolar, nodular interstitial, reticular interstitial). Individual faecal samples were tested with the Baermann migration method[23] for the presence of *A abstrusus* L1, that were identified according to morphometric and morphological features.[24] Their identity was genetically confirmed using species-specific PCRs on both faeces and pharyngeal swabs.[25]

Eligible cats were then enrolled in the study according to the inclusion/exclusion criteria as follows:

a. Inclusion criteria
   - copromicroscopic detection of *A abstrusus* L1, followed by the genetic confirmation of their molecular identity, and
   - presence of respiratory signs and/or thoracic radiographic alterations (sum of CS and RS greater than or equal to 4 points; see above).

b. Exclusion criteria
   - animals that received a macrocyclic lactone or any other anthelmintic treatment with a systemic biodistribution within the two months before the testing, or
   - animals affected by concomitant parasitic respiratory infections (eg, *Capillaria aerophila*, *Troglostrongylus brevior*) or other diseases, or
   - cats resulted positive to FeLV and/or FIV tests or,
   - cats weighing less than 0.5 kg.

Cats that fulfilled the above criteria were enrolled and allocated randomly in two study groups, a treatment group (T) and a control group (C) left untreated. The allocation was carried out according to a randomisation list created by the validated program RANCODE Professional V. 3.6 by *IDV Datenanalyse und Versuchsplanung*.

Cats were subjected to a series of clinical, laboratory and parasitological examinations according to the schedule of events reported in table 1, to assess the efficacy of the treatments in comparison with the persistence of the parasitism. The pretreatment evaluation (ie, day −3/−1) was considered as the baseline. Radiographs were evaluated by a

Table 1 Study schedule

Visit	Screening	Baseline	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
Study Day	**−15/−4**	**−3/−1**	**0**	**3***	**7***	**14***	**28*§**	**42***	**56***	**70***
Informed consent	●									
Inclusion/exclusion	●									
Randomisation	●									
Medical history	●									
Clinical examination	●	●	●	●	●	●	●	●†	●†	●†
Radiographic examination	●	●			●	●	●	●†	●†	●†
Complete blood count and serum chemistry		●				●	●	●†	●†	●†
Qualitative Baermann	●									
Quantitative Baermann		●					●	●†	●†	●†
PCR faeces	●	●					●	●†	●†	●†
PCR pharyngeal swabs		●					●			
Parasiticide treatment		-	●			●	●‡	●‡		

*A visit window of ±2 days was permitted for Visit 2 to follow-up.
†For cats belonging to the Treatment group.
‡Rescue treatment for cats belonging to the Control group.
§End of the study for cats belonging to the Control group.

board-certified veterinary radiologist blinded to the groups.

Cats of group T were treated two weeks apart (ie, at day 0 and day 14±2) with an application on the neck skin at the base of the skull of the topical formulation containing emodepside 2.1% w/v and praziquantel 8.6% w/v; the resulting dose range was 3–6 mg/kg bodyweight of emodepside and 12–24 mg/kg bodyweight of praziquantel. A rescue treatment with the same formulation was given to all control cats at the end of the study (ie, day 28±2) (table 1).

A thorough post-treatment clinical assessment was performed in order to detect any potential adverse event.

The assessment of treatment response was based on clinical evaluation as the primary efficacy criterion and parasitological efficacy as the secondary end point. Clinical response was assessed through an evaluation of CS and RS at baseline and at each follow-up visit after therapy. Presence/absence of clinical signs, clinicopathological abnormalities and radiographic patterns was also assessed at each control visit.

The value of larvae per gram (LPG) of faeces at the Baermann's test postbaseline at day 28±2 was calculated as per cent reduction according to the formula:

$$Reduction(\%) = 100 \times \frac{(Mean\ LPG\ at\ Baseline - Mean\ LPG\ at\ day\ 28)}{Mean\ LPG\ at\ Baseline}$$

Postbaseline LPG was compared with the control group for non-inferiority at day 28±2. The values of postbaseline LPG at day 42±2, day 56±2 and day 70±2 were compared with postbaseline values at day 28±2 to evaluate the persistence of negativity of larval shedding after the treatment.

All procedures, with the exception of the radiographic examinations, were not blinded.

Statistical analysis

A statistical analysis was performed using the software GraphPad Prism V.6.01. All data were evaluated using standard descriptive statistics and reported as mean±*sd* or as median and range (minimum-maximum), based on their distribution. Normality was checked graphically or using the D'Agostino Pearson test. Variables sequentially collected were compared using repeated measure one-way analysis of variance or a Friedman test and a post hoc test (Holm-Sidak test or Dunn test) was performed. Data obtained from both groups were compared using unpaired t test or Mann-Whitney test, while Fisher's exact test was used to compare categorical variables. A P value<0.05 was considered significant.

Results

Seventeen cats met the inclusion criteria and were enrolled in the study, that is, eight in the T group and nine cats in the C group.

At baseline, no differences were observed between the groups in terms of CS, RS and LPG count. Furthermore, the presence of clinical signs, laboratory and radiographic abnormalities was observed during pretreatment evaluation in both groups without any significant difference. History and physical findings, laboratory alterations and radiographic features at baseline of the cats enrolled are summarised in table 2.

Clinical efficacy

The median scores of cats belonging to the T group decreased constantly until the end of the study (table 3). A significant postbaseline reduction of the CS and RS was observed at day 28±2 (P_{CS} <0.01; P_{RS} <0.05), with a null overall score (ie, CS +RS) at the end of the study. No postbaseline differences were observed in the C group, that is, CS and RS at day 28±2 similar to those observed at the baseline (figure 1).

When compared with control cats, a significant reduction in the number of cats presenting with adventitious breath sounds, such as increased bronchovesicular lung sounds, crackles and wheeze

Table 2 Frequency of clinical, laboratory and radiographic abnormalities in 17 cats infected with *Aelurostrongylus abstrusus* at referral

Clinical signs and history complaints	N (%)	Laboratory abnormalities	N (%)	Radiographic patterns	N (%)
Coughing	13 (76.5)	Hypoalbuminaemia	9 (52.9)	Interstitial and bronchial	10 (58.8)
Bronchovesicular lung sounds	12 (70.6)	Anaemia	5 (29.4)	Interstitial	4 (23.5)
Tachypnoea	10 (58.8)	Increased ALT	5 (29.4)	Bronchial	1 (5.9)
Crackles	7 (41.2)	Neutrophilia	3 (17.6)	Alveolar and bronchial	1 (5.9)
Decreased activity	6 (35.3)	Increased AST	1 (5.9)	Alveolar and interstitial	1 (5.9)
Pale mucosae	5 (29.4)	Lymphocytosis	1 (5.9)		
Oculonasal discharge	4 (23.5)	Lymphopenia	1 (5.9)		
Wheezes sound	4 (23.5)	Eosinophilia	1 (5.9)		
Hyporexia	3 (17.6)	Eosinopenia	1 (5.9)		
Weight loss	1 (5.9)	Monocytosis	1 (5.9)		
Sneezing	1 (5.9)				

ALT, alanine aminotransferase; AST, aspartate aminotransferase; N, number of observations.

sounds, was observed in the T group at day 14±2 ($p<0.05$). Even if not statistically significant, hyporexia, lethargy, ocular and/or nasal discharge and pallor of the mucosae disappeared earlier (ie, day 14±2) in treated cats compared with those left untreated. Moreover, in treated animals, a significant reduction in number of cats with cough ($p<0.01$) and tachypnoea ($p<0.05$) was observed at day 28±2 compared with untreated cats. From the same day (ie, 28±2) onwards, a complete remission of clinical signs was observed in all cats receiving emodepside/praziquantel, while cats belonging to the C group were still symptomatic (table 4).

Besides the reduction in severity of RS, at day 28±2 a significant decrease in radiographic lesions in treated cats, compared with the control group, was recorded ($p<0.01$). However, a complete recovery from radiographic signs, in all treated animals was achieved at day 70±2 (table 4).

No differences were detected in cats of both groups presenting with haematobiochemical abnormalities, although a resolution of inflammatory leucogram was achieved earlier (ie, day 28±2) in treated cats compared with control cats (table 4). Furthermore, in comparison with baseline values, red blood cell count and albumin levels significantly increased at day 70±2 in cats that received the treatment ($p<0.01$) (figure 2).

No adverse effects were recorded after the application of topical emodepside/praziquantel.

Parasitological efficacy

The mean LPG count of cats belonging to the T group was 97.5 (*sd*±61.1) at baseline versus 0 observed at day 28±2, with a postbaseline reduction of 100 per cent ($p<0.001$). Baermann's test was also negative until the end of the study, that is, at day 42±2, day 56±2 and day 70±2. The mean LPG count of the C group was 95.0 (*sd*±43.7) at baseline versus 140.0 (*sd*±101.2) at day 28±2, with a postbaseline LPG increase of 47.4%.

Discussion

Emodepside belongs to the cycloocta depsipeptide class and it is effective against a wide variety of nematodes in companion animals.[22] The efficacy of emodepside/praziquantel spot-on solution against the cat lungworm *A abstrusus* has already been shown in natural and experimental studies,[12 18 22] that resulted in the label claim in 2016.[26] The claim was granted based on larval count reductions and worm count reduction as efficacy criterion in two laboratory dose confirmation studies and one field study.[18 22] The present data further confirm both efficacy and safety of this parasiticide formulation in guaranteeing the cessation of larval shedding in naturally infected cats.

Furthermore, the two-treatment regimen was able to assure the complete remission of both clinical and radiographic signs within 10 weeks in all treated animals. A significant improvement of clinical pictures was recorded within one month after the first administration but a partial improvement (eg, increase

Table 3 Clinical and radiographic scores of cats belonging to the two groups during the study

	BL	Day 3	Day 7	Day 14	Day 28	Day 42	Day 56	Day 70
Clinical score								
Group T	3 (1–6)	2.5 (1–7)	1.5 (0–4)	1 (0–3)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Group C	6 (2–10)	5 (2–10)	5 (2–10)	5 (2–10)	5 (2–10)			
P value	*ns*	*ns*	<0.01	<0.01	<0.001			
Radiographic score								
Group T	3 (1–4)	-	2 (1–4)	1.5 (0–2)	0 (0–2)	0 (0–1)	0 (0–1)	0 (0–0)
Group C	2 (1–5)		3 (1–5)	3 (1–5)	3 (1–5)			
P value	*ns*	-	*ns*	*ns*	<0.05			

Values are expressed as median with minimum and maximum in brackets. Statistical significance is shown (Mann-Whitney test).
BL, baseline; *ns*, not significant.

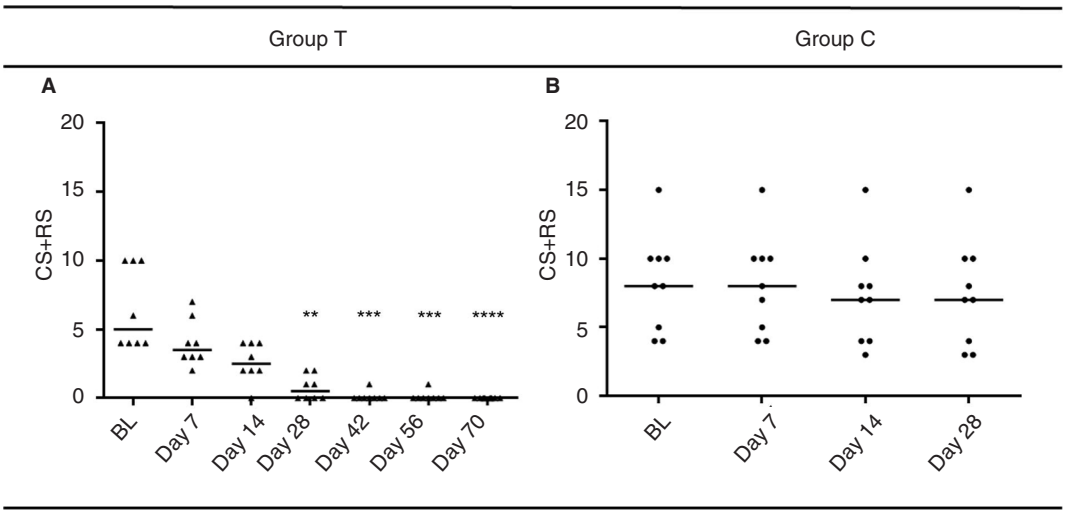

Figure 1 Sum of clinical scores (CS) and radiographic scores (RS) (overall score), after treatment (a) and in control (b) groups, recorded during the study. Post-treatment values are compared with the baseline (Friedman test). Each triangle in box *a* and each dot in box *b* represent the overall score of single cats. Each horizontal line represents the median values for the overall score. ** p<0.01; *** p<0.001; **** p<0.0001. BL, baseline.

of food intake, disappearance or reduction of oculonasal discharge and adventitious breath sounds) was already observed two weeks after the first treatment. The second application of emodepside/praziquantel guaranteed the disappearance of all clinical signs, the recovery from radiographic lesions and a parasitological negativisation. Therefore, an apparent improvement of the clinical picture after one dosing only, should not encourage a single application regimen for treating cat aelurostrongylosis with this parasiticide. Indeed, although the single treatment resulted in a reduction of larval shedding observed by the Baermann test,[18 22] two applications of Profender spot-on given two weeks apart may result in a reduction of 100 per cent of adult parasitic burden in the lungs.[22]

The blood tests in feline aelurostrongylosis are usually within normal limits or suggestive of a non-specific inflammatory status.[12 16] An altered white blood cell count secondary to inflammation has been occasionally reported previously on lungworm infections.[11 12 16] This information fit with data here obtained, as an inflammatory leucogram was observed only in four cats. However, a normalisation of leucogram pattern was observed in treated cats, in contrast with those left untreated.

Interestingly, even though increased lymphocytes secondary to an antigenic stimulation is an uncommon finding in cats,[27] massive lymphocytosis has been described in experimentally induced *A abstrusus* infection[16] and a persistent lymphocytosis, associated with secondary morphological changes consistent with lymphocyte activation, was described in two cats here. On the other hand, a transient lymphocytosis without morphological changes, observed in some of the study cats here could be related to an adrenaline response.[27] Therefore, the examination of the lymphocyte morphology in blood smear is a crucial step in differentiating the possible causes of lymphocytosis in cats with *A abstrusus* infection.

Moreover, although parasitism is considered a common cause of eosinophilia[28] and, accordingly, an increased eosinophil count has been reported in natural or experimental aelurostrongylosis,[15 16 28] a persistent eosinophilia was detected here in two cats only. Therefore, as already observed,[12] eosinophil counts should not be considered as a diagnostic indication for lungworms and the absence of eosinophilia should not rule out aelurostrongylosis from a differential diagnosis.

Anaemia and hypoalbuminaemia appear suggestive in feline aelurostrongylosis. In particular, anaemia secondary to the chronic inflammation is a common condition associated with *A abstrusus* infection[12 16 29] and albumin is one of the main negative acute phase proteins, with mild reduced levels commonly observed in chronic inflammatory diseases.[30] In this study, values of these analytes significantly increased in eight weeks after the second application of emodepside/praziquantel, providing a long-term parameter for the estimation of inflammatory status and clinical course. Finally, increased liver enzymes were recorded in cats of both groups and, in those receiving treatment, AST and/or ALT normalised in two weeks after the second application of emodepside/praziquantel spot-on. This suggests a potential bystander hepatic injury in cats infected by *A abstrusus* as increased liver enzymes have been occasionally described in feline lower airways disease[15] and lungworm infections.[12]

To date, the Baermann method is the standard technique to diagnose *A abstrusus* infections.[2] Nonetheless, false-negative results are possible[9] and the differentiation from other nematodes, such as *T brevior*, requires specific expertise and skills.[31 32] Therefore, the follow-up of cats affected by *A abstrusus* should not consist only of repeated copromicroscopy, but should encompass a careful clinical examination. In particular, the present data suggest that the clinical recovery after therapy with emodepside/praziquantel can be broken down into three phases as follows:

Table 4 Number of cats with clinical signs, laboratory alterations and radiographic patterns (ie, bronchial, alveolar, nodular interstitial and reticular interstitial patterns) associated with aelurostrongylosis.

Clinical signs	Group	BL	Day 3	Day 7	Day 14	Day 28
Cough	T	6 (75%)	6 (75%)	4 (50%)	4 (50%)	*0*
	C	7 (77.8%)	7 (77.8%)	7 (77.8%)	7 (77.8%)	*7 (77.8%)*
	P value	1.000	1.000	0.335	0.335	***0.002***
Tachypnoea	T	4 (50%)	3 (37.5%)	2 (25%)	1 (50%)	*0*
	C	6 (66.7%)	6 (66.7%)	6 (66.7%)	4 (44.4%)	*5 (55.5%)*
	P value	0.637	0.346	0.153	0.294	***0.029***
Pale mucous membranes	T	2 (25%)	2 (25%)	1 (12.5%)	0	0
	C	3 (33.3%)	3 (33.3%)	3 (33.3%)	2 (22.2%)	2 (22.2%)
	P value	1.000	1.000	0.576	0.470	0.470
Reduction of food intake	T	2 (25%)	1 (12.5%)	0	0	0
	C	1 (11.1%)	1 (11.1%)	1 (11.1%)	1 (11.1%)	1 (11.1%)
	P value	0.576	1.000	1.000	1.000	1.000
Reduction of the activity	T	2 (25%)	1 (12.5%)	1 (12.5%)	0	0
	C	4 (44.4%)	4 (44.4%)	4 (44.4%)	3 (33.3%)	3 (33.3%)
	P value	0.619	0.294	0.082	0.206	0.206
Oculonasal discharge	T	1 (12.5%)	1 (12.5%)	1 (12.5%)	0	0
	C	4 (44.4%)	4 (44.4%)	4 (44.4%)	4 (44.4%)	4 (44.4%)
	P value	0.294	0.294	0.294	0.082	0.082
Adventitious breath sounds	T	6 (75%)	6 (75%)	5 (62.5%)	*3 (37.5%)*	*0*
	C	8 (88.9%)	8 (88.9%)	8 (88.9%)	*8 (88.9%)*	*7 (77.8%)*
	P value	0.576	0.576	0.294	***0.049***	***0.002***
Anaemia	T	2 (25%)	-	-	2 (25%)	0
	C	3 (33.3%)	-	-	1 (11.1%)	0
	P value	1.000			0.576	1.000
Inflammatory response leucogram	T	2 (25%)	-	-	2 (25%)	0
	C	2 (22.2%)	-	-	2 (22.2%)	3 (33.3%)
	P value	1.000			1.000	0.205
Stress response leucogram	T	1 (12.5%)	-	-	1 (12.5%)	2 (25%)
	C	0	-	-	0	0
	P value	0.470			0.470	0.205
Excitement response leucogram	T	1 (12.5%)	-	-	0	0
	C	1 (11.1%)	-	-	0	0
	P value	1.000			1.000	1.000
Low albumin levels	T	3 (37.5%)	-	-	2 (25%)	1 (12.5%)
	C	6 (66.7%)	-	-	5 (55.5%)	3 (33.3%)
	P value	0.346			0.334	0.576
Increased ALT/AST	T	4 (50%)	-	-	0	2 (25%)
	C	2 (22.2%)	-	-	1 (11.1%)	1 (11.1%)
	P value	0.334			1.000	0.576
Presence of at least one radiographic sign	T	8 (100%)	-	8 (100%)	5 (62.5%)	*3 (37.5%)*
	C	9 (100%)	-	9 (100%)	9 (100%)	*9 (100%)*
	P value	1.000		1.000	0.082	***0.009***

P values are shown (Fisher's exact test). Cats of Group T=8; cats of Group C=9. Percentages in brackets. Bold values denote statistical significance.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BL, baseline.

(*i*) *early phase*, within two weeks from the first administration, characterised by a significant reduction of bronchovesicular lung sounds, crackles and wheezes, the disappearance of oculonasal discharge and the increase of both appetite and activity; (ii) *middle phase*, two weeks after the second administration, when a recovery from cough and tachypnoea and the resolution of radiographic lesions can be observed in the vast majority of cats. When presents at the time of diagnosis, also inflammatory leucogram and increased liver enzymes disappear in this phase; (*iii) late phase*, characterised by the improvement of anaemia and hypoalbuminaemia, occurring about two months after the second treatment, probably due to a progressive resolution of the inflammation. Further studies, using emodepside and other parasiticides, are warranted to confirm these preliminary data.

From a clinical point of view, each animal affected by aelurostrongylosis should undergo a thorough physical examination, a radiographic study of the thorax and a haematobiochemical profile. After diagnosis and subsequent first parasiticide treatment, a proposed timeline should be based on fortnightly controls, encompassing physical examination and Baermann test, until complete clinical recovery. The radiographic follow-up should be performed one month after the diagnosis, and then on a monthly basis until the complete resolution of radiographic alterations. As blood sampling

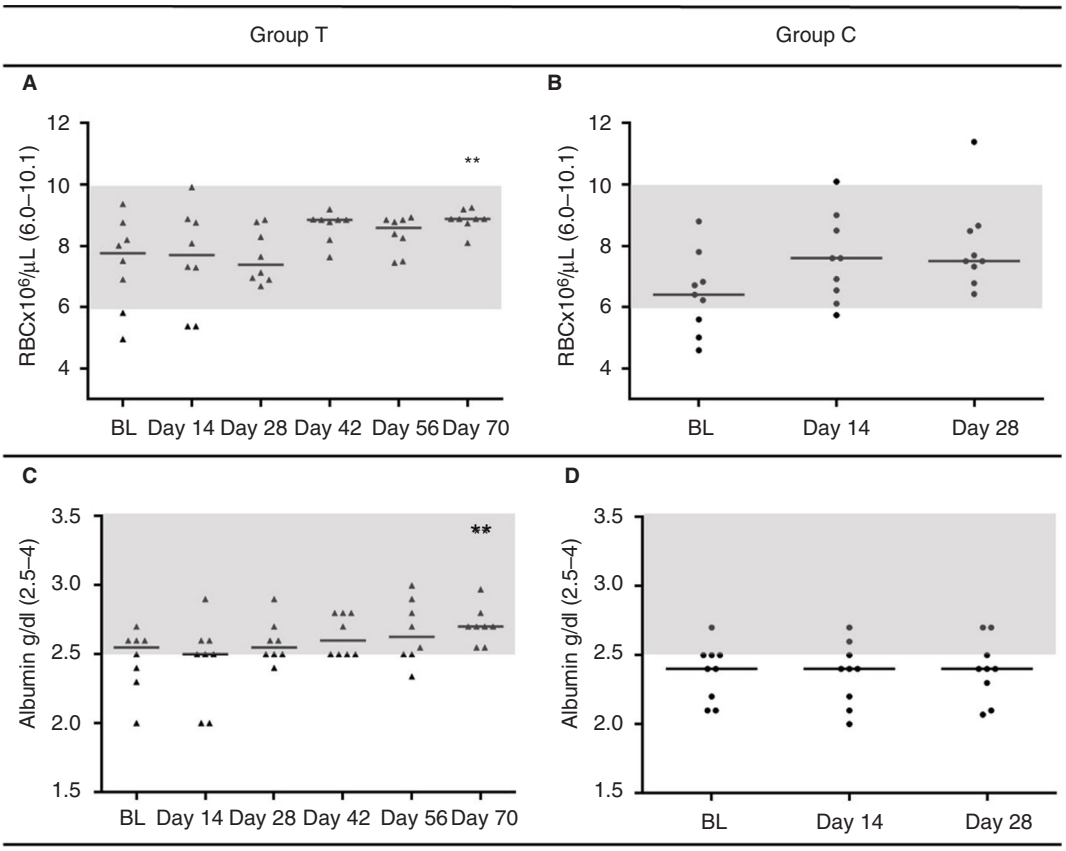

Figure 2 Red blood cells (a, b) and albumin (c, d) levels, after treatment (a, c) and in control group (b, d) recorded during the study. Post-treatment values are compared with the baseline (Friedman test). Grey area represents normal values. Each triangle in the boxes *a* and *c* and each dot in the boxes *b* and *d* represent singles values of red blood cell count and albumin level of each cat. Each horizontal line represents the median values. ** p<0.01; BL, baseline; RBC, red blood cells.

can be difficult in cats because of their natural reluctance to be restrained, laboratory test could be delayed by up to eight weeks after the second administration of emodepside/praziquantel in those cats presenting at the diagnosis with laboratory signs of chronic inflammation (eg, anaemia, low albumin levels). If an inflammatory leucogram pattern and/or a mild increase of liver enzymes are observed at the time of diagnosis, blood test is advisable four weeks after the first visit.

This study has some potential limitations. All the procedures, with the exception of the radiographic interpretation, were not blinded. Also, even though not statistically significant, the apparent baseline imbalances in clinical severity between study groups may lead to an inaccurate evaluation of the treatment response. Finally, because of the relatively small population size, a failure to detect more specific differences between the groups cannot be excluded. Nonetheless, the efficacy of emodepside/praziquantel in treating natural cat aelurostrongylosis is confirmed in controlled field conditions. Two applications of this spot-on solution two weeks apart led to parasitological negativisation and to a general improvement of the clinical picture, *via* the early reduction of clinical and radiographic signs, followed by a complete remission of clinicopathological abnormalities. Further investigations on the clinical efficacy of emodepside (and other parasiticides) in treating cat lungworms in larger animal cohorts are, however, truly warranted.

Acknowledgements The authors thank Bayer Animal Health, Germany, for their support to this study. The authors also thank Raffaella Iorio for collaborating with the present study.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests RS and FP are currently employed by Bayer Animal Health.

Patient consent for publication Not required.

Ethical statement This work involved client-owned animals only and was performed with the full informed consent of the owners or legal guardians of all animals described in this work for the procedure undertaken. All the information was derived from necessary clinical interventions and the requirements of the Italian Legislative Decree 04/03/2014 n. 26 (Implementation of the Directive 2010/63 / EU on the protection of animals used for scientific purposes) were followed. No animals or humans are identifiable within this publication, and therefore additional informed consent for publication was not required.

Data availability statement All data relevant to the study are included in the article.

ORCID iD
Paolo Emidio Crisi http://orcid.org/0000-0001-5993-0059

References

1 Anderson RC. The superfamily Metastrongyloidea. In: Anderson RC, ed. Nematode parasites of vertebrates. their development and transmission. UK, Wallingford: C.A.B. International, 2000: 163–4.
2 Traversa D, Di Cesare A. Diagnosis and management of lungworm infections in cats: Cornerstones, dilemmas and new avenues. *J Feline Med Surg* 2016;18:7–20.
3 Traversa D, Di Cesare A, Milillo P, *et al*. *Aelurostrongylus abstrusus* in a feline colony from central Italy: clinical features, diagnostic procedures and molecular characterization. *Parasitol Res* 2008;103:1191–6.

4 Traversa D, Lia RP, Iorio R, *et al*. Diagnosis and risk factors of *Aelurostrongylus abstrusus* (Nematoda, Strongylida) infection in cats from Italy. *Vet Parasitol* 2008;153:182–6.
5 Mircean V, Titilincu A, Vasile C. Prevalence of endoparasites in household cat (*Felis catus*) populations from Transylvania (Romania) and association with risk factors. *Vet Parasitol* 2010;171:163–6.
6 Capári B, Hamel D, Visser M, *et al*. Parasitic infections of domestic cats, *Felis catus*, in western Hungary. *Vet Parasitol* 2013;192:33–42.
7 Naylor JR, Hamilton JM, Weatherley AJ. Changes in the ultrastructure of feline pulmonary arteries following infection with the lungworm *Aelurostrongylus abstrusus*. *Br Vet J* 1984;140:181–90.
8 Dennler M, Bass DA, Gutierrez-Crespo B, *et al*. Thoracic computed tomography, angiographic computed tomography, and pathology findings in six cats experimentally infected with *Aelurostrongylus* abstrusus. *Vet Radiol Ultrasound* 2013;54:459–69.
9 Traversa D, Di Cesare A, Conboy G. Canine and feline cardiopulmonary parasitic nematodes in Europe: emerging and underestimated. *Parasit Vectors* 2010;3:62.
10 Febo E, Crisi PE, Traversa D, *et al*. Comparison of clinical and imaging findings in cats with single and mixed lungworm infection. *J Feline Med Surg* 2019;21:581–9.
11 Grandi G, Calvi LE, Venco L, *et al*. *Aelurostrongylus abstrusus* (cat lungworm) infection in five cats from Italy. *Vet Parasitol* 2005;134:177–82.
12 Crisi PE, Aste G, Traversa D, *et al*. Single and mixed feline lungworm infections: clinical, radiographic and therapeutic features of 26 cases (2013-2015). *J Feline Med Surg* 2017;19:1017–29.
13 Gavrilović P, Jovanović M, Gavrilović A, *et al*. Fatal aelurostrongylosis in a kitten in Serbia. *Acta Parasitol* 2017;62:488–91.
14 Soares C, Cardoso M, Mestre A, *et al*. Case report: Severe and progressive bronchopneumonia by *Aelurostrongylus abstrusus* in an adopted stray cat from Portugal. *J Parasit Dis* 2017;41:976–80.
15 Foster SF, Martin P, Allan GS, *et al*. Lower respiratory tract infections in cats: 21 cases (1995-2000). *J Feline Med Surg* 2004;6:167–80.
16 Schnyder M, Di Cesare A, Basso W, *et al*. Clinical, laboratory and pathological findings in cats experimentally infected with *Aelurostrongylus abstrusus*. *Parasitol Res* 2014;113:1425–33.
17 Lacava G, Zini E, Marchesotti F, *et al*. Computed tomography, radiology and echocardiography in cats naturally infected with *Aelurostrongylus abstrusus*. *J Feline Med Surg* 2017;19:446–53.
18 Traversa D, Milillo P, Di Cesare A, *et al*. Efficacy and safety of emodepside 2.1%/praziquantel 8.6% spot-on formulation in the treatment of feline aelurostrongylosis. *Parasitol Res* 2009;105 Suppl 1:83–90.
19 Traversa D, Di Cesare A, Milillo P, *et al*. Efficacy and safety of imidacloprid 10%/moxidectin 1% spot-on formulation in the treatment of feline aelurostrongylosis. *Parasitol Res* 2009;105(Suppl 1):S55–62.
20 Giannelli A, Brianti E, Varcasia A, *et al*. Efficacy of Broadline® spot-on against *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* lungworms in naturally infected cats from Italy. *Vet Parasitol* 2015;209:273–7.
21 Knaus M, Chester ST, Rosentel J, *et al*. Efficacy of a novel topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel against larval and adult stages of the cat lungworm, *Aelurostrongylus abstrusus*. *Vet Parasitol* 2014;202:64–8.
22 Böhm C, Wolken S, Schnyder M, *et al*. Efficacy of Emodepside/Praziquantel Spot-on (Profender®) against adult *Aelurostrongylus abstrusus* Nematodes in Experimentally Infected Cats. *Parasitol Res* 2015;114(Suppl 1):S155–64.
23 Euzéby J. Diagnostic expérimental des helminthoses animales: travaux pratiques d'helminthologie vétérinaire/. Paris: Informations techniques des services vétérinaires français, 1981.
24 Traversa D, Di Cesare A. Feline lungworms: what a dilemma. *Trends Parasitol* 2013;29:423–30.
25 Traversa D, Iorio R, Otranto D. Diagnostic and clinical implications of a nested PCR specific for ribosomal DNA of the feline lungworm *Aelurostrongylus abstrusus* (Nematoda, strongylida). *J Clin Microbiol* 2008;46:1811–7.
26 European Medicines Agency. Profender: EPAR - product information. Available: www.ema.europa.eu/en/medicines/veterinary/ [Accessed 28 Sep 2018].
27 Avery AC, Avery PR. Determining the significance of persistent lymphocytosis. *Vet Clin North Am Small Anim Pract* 2007;37:267–82.
28 Randolph JF, Erb HN, Reiter S, Center SA. Eosinophilia in the cat: a retrospective study of 312 cases (1975 to 1986). *J Am Anim Hosp Assoc* 1990;26:349–58.
29 Mahaffey MB. Radiographic-pathologic findings in experimental Aelurostrongylus abstrusus infection in cats. *Veterinary Radiology* 1979;20:81.
30 Cerón, JJ, Eckersall PD, Martýnez-Subiela S. Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Vet Clin Pathol* 2005;34:85–99.
31 Brianti E, Giannetto S, Dantas-Torres F, *et al*. Lungworms of the genus *Troglostrongylus* (strongylida: Crenosomatidae): neglected parasites for domestic cats. *Vet Parasitol* 2014;202:104–12.
32 Di Cesare A, Veronesi F, Grillotti E, *et al*. Respiratory nematodes in cat populations of Italy. *Parasitol Res* 2015;114:4463–9.