



Efficacy of two topical combinations containing emodepside plus praziquantel, and emodepside plus praziquantel plus tigolaner, for the treatment of troglostrongylosis in experimentally infected cats



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ARTICLE INFO

Keywords:

Cat
Emodepside
Lungworms
Treatment
Troglostrongylus brevior

ABSTRACT

Feline troglostrongylosis caused by *Troglostrongylus brevior* is increasingly reported in European countries. Although the disease can be severe and potentially life-threatening, especially in kittens and young cats, effective treatment options are still limited. Two administrations of emodepside 2 weeks apart have shown promising results for the treatment of *T. brevior* infection in single cases and in a field trial. Therefore, the present study has been conducted to evaluate the efficacy of two spot-on combinations containing emodepside (i.e. 2.14% w/v emodepside and 8.58% w/v praziquantel - Profender®, and 2.04% w/v emodepside, 8.14% w/v praziquantel and 9.79% w/v tigolaner - Felpreva®) in the treatment of troglostrongylosis under experimental conditions. Twenty-four cats were experimentally infected with *T. brevior* and randomly assigned to one of three groups of eight cats each, i.e. (i) Group 1 (G1) left untreated, (ii) Group 2 (G2) receiving Profender® on Days 28 and 44, and (iii) Group 3 (G3) receiving Felpreva® on Day 28 and Profender® on Day 44. Doses corresponded to the minimum effective dose of 0.140 and 0.148 ml/kg body weight, for Profender® and Felpreva®, respectively. The primary efficacy criterion was the number of viable adult *T. brevior* counted at necropsy conducted between Days 69 and 72. The fecal shedding of first-stage larvae (L1) was also assessed. L1 of *T. brevior* were detected in samples from all cats within 20 days post-infection. At necropsy, 4 of 8 G1 cats harbored adult *T. brevior*, while no adult *T. brevior* worms or other development stages were recovered from any of the G2 and G3 cats. The primary efficacy criterion was not evaluated as the worm counts in G1 did not meet VICH guideline requirements. After the first treatment (Day 28), most G2 and G3 cats were negative at the Baermann examination. After the second treatment (Day 44), L1 were found in two cats from G2 on Day 49 and in one G3 cat on Day 51. No adverse events occurred in G2 and G3 cats. These results indicate that two applications of emodepside spot-on given 2 weeks apart represent a safe and efficacious treatment regime against troglostrongylosis.

1. Introduction

The parasitic nematode *Troglostrongylus brevior* (Metastrongyloidea: Crenosomatidae) is a cause of bronchopneumonia in domestic cats across Europe, in particular in Mediterranean countries and Eastern territories (Morelli et al., 2021; Traversa et al., 2021). Adults of *T. brevior* reside in the airways of infected cats, specifically in the bronchi and bronchioles, where they mate. After mating, females

release eggs which hatch, then first-stage larvae (L1) are transported to the pharynx by mucociliary clearance, swallowed and reach the environment via the feces. The larvae develop to the infective third larval stage (L3) inside intermediate hosts represented by terrestrial molluscs. Cats become infected when ingesting intermediate hosts or, more frequently, paratenic hosts (Traversa et al., 2021), and there is also evidence that *T. brevior* may be transmitted from the queen to the kittens, likely via a transmammary route (Traversa et al., 2018).

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<https://doi.org/10.1016/j.crpvbd.2022.100097>

Received 16 March 2022; Received in revised form 12 July 2022; Accepted 14 July 2022

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Infected cats, especially kittens and young animals, may suffer severe and potentially fatal catarrhal bronchitis and interstitial pneumonia, characterized by coughing, sneezing, dyspnea, tachypnea, and some non-specific clinical signs (Morelli et al., 2021).

The clinical relevance of troglstrongylosis in cats and its growing geographical expansion (Diakou et al., 2015, 2017; Giannelli et al., 2017; Crisi et al., 2018; Traversa et al., 2019a; Salant et al., 2020) calls for the implementation of therapeutic options. To date, few studies have been conducted to evaluate the efficacy and safety of anthelmintics in cats infected with *T. brevior* under natural and laboratory conditions (reviewed in Traversa et al., 2021). The present article describes a study performed to investigate the efficacy of two spot-on combinations containing 2.14% w/v emodepside and 8.58% w/v praziquantel (Profender®, Vetoquinol) and 2.04% w/v emodepside, 8.14% w/v praziquantel and 9.79% w/v tigolaner (Felpreva®, Vetoquinol) in the treatment of experimental troglstrongylosis.

2. Materials and methods

2.1. Study design and animals

This was a blinded, placebo-controlled, single-site efficacy study using a randomized block design, performed in accordance with Veterinary International Conference on Harmonization Guidelines (VICH Guideline 7: “Efficacy of anthelmintics: General requirements”; VICH Guideline 9: “Guideline on Good Clinical Practice”; and VICH Guideline 20: “Efficacy of anthelmintics: Specific recommendations for felines” (EMA, 2000a, b, 2001)).

Twenty-four ($n = 24$) cats, i.e. 12 females and 12 males, were enrolled and acclimatized in the study facility at the Institute for Parasitology, University of Veterinary Medicine Hannover, Germany, for 14 days. At study inclusion on Day -1 the cats were 19–22 weeks-old and weighted 1.70–2.90 kg. Cats enrolled in the study had to be endoparasite-free. This was proved by three fecal samples examined using the combined sedimentation-flotation and Baermann technique.

Housing of the cats complied with the Directive 2010/63/EU of the European Parliament and of the Council of 22nd September 2010 on the protection of the animals used for scientific purposes. Compliance to aspects of animal welfare law was also verified according to the German Animal Protection Act and the German Welfare Regulation for Laboratory Animals, and Company Animal Welfare Commissioner. The animals were group-housed by study groups and same sex, while they were kept in individual cages for treatment and fecal sampling on the respective days. The cats were fed with standard feline diet and water was provided *ad libitum*.

2.2. Allocation and treatment

On Days -7 and -1 all cats underwent a clinical examination for inclusion and cats meeting the inclusion criteria on Day -1 were included in the study, based on a rank according to body weight within sex and assigned to one of three groups of eight cats each, i.e. Group 1 (G1) left untreated, Group 2 (G2) receiving Profender® on Days 28 and 44, and Group 3 (G3) receiving Felpreva® on Day 28 and Profender® on Day 44. Four females and four males were included in each group. Doses were administered topically by parting the fur on the cat's neck at the base of the skull and applying the spot-on directly onto the skin. Doses corresponded to the minimum effective dose of 0.140 and 0.148 ml/kg body weight, for Profender® and Felpreva®, respectively. Cats that met the following inclusion criteria were enrolled in the study: (i) acclimatization for at least 14 days; (ii) clinically healthy according to the clinical examination on Day -7 ; (iii) age > 10 weeks on Day 28; (iv) weight > 1 kg on Day 28; (v) not pregnant, not excessively fractious; (vi) negative worm egg counts at three individual fecal examinations between Day -7 and Day -1 ; (vii) not treated with macrocyclic lactones or any other drug that could have interfered with the evaluation of the products administered at least 3 months prior to study start.

2.3. Source of infective larvae and cat infection

First-stage larvae (L1) of *T. brevior* were collected from a privately owned naturally infected cat living in Italy. The donor cat had a subclinical infection and was enrolled after the informed consent form signed by the owner and the necessary authorizations to perform the activities. Feces were collected daily from the litter box of the cat from May to November 2019, when the cat was monitored daily for health and welfare status. Breeding, management and infection of intermediate hosts, i.e. snails of the species *Cornu aspersum*, were conducted as described in previous similar studies (Di Cesare et al., 2013). Snails were purchased from a farm breeding molluscs intended for human consumption and kept in *vivaria* under controlled conditions of lighting, temperature of ~ 24 – 25 °C and humidity of $\sim 80\%$ and fed *ad libitum* with vegetables for the whole duration of the study. Before experimental infection of the snails with *T. brevior*, a sample of $\sim 10\%$ of the farmed snails were examined microscopically and subjected to a multiplex PCR to exclude the presence of natural infections with feline metrastrongyloids (Di Cesare et al., 2015). All the remaining snails were each infected with 500 L1 of *T. brevior* as previously described (Di Cesare et al., 2013; Morelli et al., 2020).

Infective L3 were obtained on Day 0 and processed as follows. Snails were artificially digested as described (Morelli et al., 2020; Traversa et al., 2022). The digested material was then filtered using a 200 μm sieve and centrifuged at $300 \times g$ for 10 min. The sediment was resuspended in tap water, centrifuged again, pooled, and shaken to have a larval suspension set on a magnetic stirrer with heating plate maintained at 40 °C. The mean number of L3 in 0.1 ml of suspension was calculated by smearing corresponding aliquots onto glass slides. Based on these data, infection doses with an inoculum of ~ 100 L3 were prepared.

Cats were anesthetized with a combined intramuscular injection of 0.08 ml/kg BW Domitor® (1 mg/ml medetomidine HCl, Zoetis, Berlin, Germany) and 0.075 ml/kg BW Ketamin 10%® (100 mg/ml ketamine HCl, WDT). To prevent vomiting or regurgitation, cats received 0.06 ml/kg BW Emeprid® IM (5 mg/ml, metoclopramide HCl, CEVA) 15 min before inserting a stomach tube for the inoculation. The infection dose containing ~ 100 L3 was administered directly into the stomach *via* a syringe, then the tube was flushed with tap water and pulled out after having verified that no inoculum remained in the tube. All cats were observed for vomiting or regurgitation directly after inoculation for up to 30 (± 10) min post-inoculation.

2.4. Health observations

The health status of cats was observed daily during the acclimatization and for the whole duration of the study until necropsy. All cats underwent an extensive clinical examination for study inclusion by a veterinarian on Days -7 and -1 . Clinical assessments were conducted for cats in G2 and G3 pre-treatment on Days 28 and 44 as well as 4 h and 24 h after each treatment to carefully observe them for any adverse events. All cats were assessed for changes in respiratory rate and sound by auscultation prior to inoculation on Day -1 and on Days 7, 14, 21, 27, 35, 41, 49, 55, 63 and before necropsy (Days 69–72).

2.5. Parasitological examinations

Detection of patency was evaluated every other day between Days 18 and 28 in all study groups using quantitative Baermann examination as previously described (Ambrosi, 1995). Further individual fecal samples for quantitative examination were collected from all cats on consecutive days: Days 35–37, Days 42 and 43, Days 49–51 and Days 63–65. Larvae were counted and calculated as number of larvae per gram feces (LPG).

2.6. Necropsy

Cats were humanly euthanized between Days 69–72 by sedation with a combination of intramuscular administered medetomidine 0.08 mg/kg

BW (0.08 ml/kg BW of Domitor®) and ketamine 7.5 mg/kg BW (0.075 ml/kg BW of Ketamin® 10%), followed by intravenous administration of pentobarbital 130 mg/kg BW (0.26 ml/kg BW of Euthadorm®). The thorax of each cat was opened to remove lungs, trachea and heart *in toto*. Then heart and trachea were carefully separated, and the lungs were checked by dissecting piece by piece under a stereomicroscope. Recently dead intact nematodes were considered as viable worms, while fragments were counted only if the anterior or posterior end was present. If the number of anterior ends was greater than the number of posterior ends, only the anterior ends were used to calculate the total number of worms and *vice versa*.

2.7. Efficacy criteria

Adequacy of infection was considered if ≥ 5 adults of *T. brevior* were found in ≥ 6 control cats. The primary efficacy endpoint to evaluate if Profender® and Felpreva® were efficacious against adult *T. brevior* was the number of viable adult parasite worms retrieved at necropsy. The efficacy percentage was calculated as below using geometric mean (GM) as recommended in VICH GL7 (EMA, 2000a):

$$\% \text{ Efficacy (Reduction)} = (N2 - N1)/N2 \times 100$$

where N1 is the GM count of *T. brevior* for Group 2 (Profender®) or Group 3 (Felpreva®) and N2 is the GM count of *T. brevior* for Group 1 (control).

A descriptive statistical analysis for number of animals positive for *T. brevior* and GM worm counts per group was conducted for the parasite burdens of study groups. Further a statistical analysis was performed on the adult worm counts as well as the larvae per gram of feces of the two treatment groups in comparison to the control group.

3. Results

3.1. Inclusion criteria, health observations and safety assessment

All cats met the inclusion criteria and were enrolled. Data on clinical signs and alterations showed by the cats enrolled in each of the three groups are listed in Table 1. Three cats, two in Group 2 and one in Group 3, showed mild adverse events (each with one occasion of coughing on Day 28 or 29, recovering without treatment).

3.2. Parasitological examinations

During acclimatization all cats scored negative for any nematode eggs, larvae and adults at the qualitative copromicroscopy. Patency started at Day 20 and on Day 22 larvae were retrieved from all fecal samples, thus confirming patency in all cats. Six of the eight control cats (Group 1) shed larvae until necropsy. After the first treatment (Day 28), 6 and 4 cats of Group 2 and Group 3 shed low numbers of larvae between Days 35 and 43, while after the second treatment only two cats of Group 2 shed very low numbers of larvae on Day 49, and only a single cat of Group 3 shed a few larvae on Day 51. After Day 49, all cats of Group 2 and after Day 51 all cats of Group 3 remained negative at the microscopical examination until necropsy. Tables 2–4 provide detailed information on the larval shedding in each of the three study groups after the first and second treatment.

3.3. Adult worm count

All nematode specimens collected from the necropsied cats were identified as *T. brevior* and counted. Statistical efficacy calculation was not performed because the nematode counts in Group 1 control cats did not meet adequacy of infection, i.e. at least six infected cats in the control group. Indeed, only four of the eight animals harbored either viable or recently dead, intact or fragments of adult *T. brevior*, with numbers varying between 10 and 42 worms. In two of these cats, living larvae and

Table 1
Clinical signs observed in study cats

Group	Animal ID	Day	Clinical signs
1 (Untreated control)	6331	-7 ^a ; -1	Slightly underweight
	6535	-7 ^a	Slightly underweight
	6577	-1	Abdomen strained
	6598	-7 ^a	Slightly underweight
		41; 49	Sniffing
		55	Deepened respiratory sound and sniffing
2 (Profender®)	6577	70	Deepened respiratory sounds
	6344	-1	Slightly underweight
	6345	29	Coughing
		35	Deepened respiratory sound
3 (Felpreva®)	6554	35; 55; 70	Deepened respiratory sound
	6585	28	Coughing
	6550	-1	Small trace of blood on the anus after taking body temperature
		28	Coughing
		41; 70	Sniffing
	6434	35; 69	Deepened respiratory sound
		41; 55	Sniffing
	6626	21; 63	Deepened respiratory sound
6674	35	Deepened respiratory sound and sniffing	
	49	Sniffing	

^a Due to extended acclimatization period, Day -7 activities took place 14 days before inoculation.

Table 2
Fecal larval counts observed in pre-treatment cats

	Day 18/19	Day 20	Day 22	Day 24	Day 26	Day 28
No. of cats shedding larvae	0	3	24	24	22	24
Minimum LPG	0	0	1	3	0	27
Maximum LPG	0	0.2	51.2	261	1219	1206
Arithmetic mean	0	0.03	22.55	91.92	246.00	299.00

Abbreviation: LPG, larvae per gram of feces.

eggs of *T. brevior* were also observed. No adult *T. brevior* worms or other development stages were detected in any of cats treated with Profender® or Felpreva®.

3.4. Statistical analysis

For the parameter “worm count” with the non-parametric Wilcoxon-Mann-Whitney test (alpha = 0.025 one-sided, test on superiority), a large superiority of the Profender®-treated as well as for the Felpreva®-treated group *versus* the control group was observed and a small to medium sized superiority can be proven (lower bound of the Mann-Whitney test (LB-MW) = 0.5768, i.e. > 0.50 - the benchmark for superiority). Further, for the parameter “larvae per gram of feces” with the non-parametric Wilcoxon-Mann-Whitney test (alpha = 0.025 one-sided, test on superiority) a large superiority of the Profender®- and the Felpreva®-treated groups *versus* the control group was observed and proven for all days (LB-MW \geq 0.6448, i.e. > 0.50 - the benchmark for superiority).

4. Discussion

Efficacious treatment options for cat troglstrongylosis are of high relevance in feline practice, as *T. brevior* is an emerging lungworm often causing a life-threatening bronchopneumonia and permanent damages in kittens and young animals (Di Cesare et al., 2014; Cavalera et al., 2018; Morelli et al., 2021). Cats with clinical signs of troglstrongylosis need treatment and the health status must be constantly and strictly monitored. Moreover, it is crucial to treat effectively also subclinically infected

Table 3
Fecal larval counts observed after the first treatment (Day 28) in cats included in Groups 1–3

Group		Day 35	Day 36	Day 37	Day 42	Day 43
1 (Untreated control)	No. of cats shedding larvae	8	8	8	7	8
	Minimum LPG	13.8	32.6	9	0	0.4
	Maximum LPG	3255	1011	594	1464	1590
	Arithmetic mean	444.15	251.40	171.78	345.35	490.35
2 (Profender®)	No. of cats shedding larvae	3	1	0	0	1
	Minimum LPG	0	0	0	0	0
	Maximum LPG	42	0.4	0	0	1
	Arithmetic mean	5.30	0.05	0	0	0.13
3 (Felpreva®)	No. of cats shedding larvae	2	1	0	2	0
	Minimum LPG	0	0	0	0	0
	Maximum LPG	105	4.4	0	1.8	0
	Arithmetic mean	13.18	0.55	0	0.40	0

Abbreviation: LPG, larvae per gram of feces.

Table 4
Fecal larval counts observed after the second treatment (Day 44) in cats included in Groups 1–3

Group		Day 49	Day 50	Day 51	Day 63	Day 64	Day 65
1 (Untreated control)	No. of cats shedding larvae	7	7	6	7	6	6
	Minimum LPG	0	0	0	0	0	0
	Maximum LPG	609	1708	4876	669.6	3364	2016
	Arithmetic mean	171.98	367.75	1598.68	160.88	1170.88	484.50
2 (Profender®)	No. of cats shedding larvae	2	0	0	0	0	0
	Minimum LPG	0	0	0	0	0	0
	Maximum LPG	1	0	0	0	0	0
	Arithmetic mean	0.18	0	0	0	0	0
3 (Felpreva®)	No. of cats shedding larvae	0	0	1	0	0	0
	Minimum LPG	0	0	0	0	0	0
	Maximum LPG	0	0	3.6	0	0	0
	Arithmetic mean	0	0	0.45	0	0	0

Abbreviation: LPG, larvae per gram of feces.

cats because they shed larvae and are a source of infection for molluscs, regardless the presence of clinical signs.

Only a few treatment options are available for the treatment of cats infected with *T. breviar*. Two spot-on products containing eprinomectin (Broadline™ and Nexgard® Combo, Boehringer Ingelheim, Ingelheim, Germany) have been licensed in the EU market in the past years for the treatment of cat troglstrongylosis (Giannelli et al., 2015; Knaus et al., 2020; Beugnet, 2021). Other parasiticides showed their efficacy in terms of larval shedding and remission of clinical signs in cats infected with *T. breviar* either in monospecific or in mixed infection with the closely related cat lungworm *Aelurostrongylus abstrusus*. This applies to the macrocyclic lactones milbemycin oxime in single clinical cases (Crisi et al., 2017) or moxidectin in clinical reports and studies (Crisi et al., 2015, 2017) and in large trials with naturally and experimentally infected cats (Diakou et al., 2019; Traversa et al., 2022). Although the efficacy of oral fenbendazole against *T. breviar* is suggested in some guidelines (Pennisi et al., 2015), this has been not factually evaluated nor demonstrated (Morelli et al., 2021).

Regarding the cyclooptadepsipeptide emodepside, the first data were obtained under natural conditions in single cases of mixed infections with both lungworm species, *A. abstrusus* and *T. breviar*, in a purposed field trial. These studies proved that two administrations 2 weeks apart (in combination with praziquantel in Profender®) were efficacious against *T. breviar* in terms of cessation of larval shedding and clinical recovery (Di Cesare et al., 2015; Traversa et al., 2019b).

The herein presented results obtained in experimental conditions corroborate the above data, thus indicating that two administrations of Profender® 2 weeks apart or one treatment with Felpreva® followed by the administration of Profender® at a ~14-days interval are effective and safe against cat troglstrongylosis.

This study presented some limitations which, however, did not prevent to consider that the two spot-on formulations investigated are effective treatment options against *T. breviar*. In particular, the data were

not statistically analyzed because the adequacy of infection in control cats, i.e. six cats harboring worms at necropsy as per VICH GL, was not met. Nevertheless, the experimental infection was successful in all the three study groups because all cats were shedding larvae by Day 22 in accordance with the known pre-patent period of *T. breviar* (Crisi et al., 2018; Knaus et al., 2020). The study data submitted gained marketing authorization of Felpreva® for the treatment of infection with *T. breviar*.

Although it is hard to explain why some untreated cats were negative for adult *T. breviar* despite being positive for L1, some hypothesis can be drawn. The lungs were thoroughly examined, thus it is unlikely that the comparatively large worms residing in deep airways could have been missed, though it cannot be ultimately ruled out. The most likely reason is a limited lifespan of the parasites, followed by a spontaneous death triggered by immune mechanisms. In fact, in natural conditions the older the cats the lower the occurrence rate of *T. breviar*. In endemic areas troglstrongylosis is a frequent disease in cats aged ≤ 6 months, whilst it is rarer in older cats up to 2 years of age and seldom or not diagnosed in cats aged ≥ 2 years (Giannelli et al., 2017; Cavalera et al., 2018). Accordingly, as cats aged ~5–5.5 months, some anatomical and immunological drivers could have had an influence on the survival rate of *T. breviar*, with a frequent spontaneous death and elimination of worms.

Despite the negativity for adults of *T. breviar* at necropsy in half of the control cats, most of them (i.e. 6/8) continued to shed L1 until the completion of the study. Given that 8/8 and 7/8 control cats shed L1 until Day 43 and 63, respectively, it can be concluded that (i) adult nematodes survived until later phases of the study, well beyond when larval shedding had ceased in most Group 2 and Group 3 animals, and (ii) the death of adult *T. breviar* started between 6 and 9 weeks post-infection. Very few treated cats still had L1 in their feces post treatment, i.e. two in Group 2 after first or second administration, and two and one in Group 3 after first or second administration, respectively. The number of shed L1 was very low (Tables 2–4), thus it can be argued that these were larval stages which hatched from eggs released prior to the second treatment or by

decreasing worms. Accordingly, it has been shown that cats infected with *T. brevior* may shed larvae up to 10 days post-treatment with macrocyclic lactones (Cavalera et al., 2019; Traversa et al., 2022). As adult worms reside in the deep airways, clearance of the lungs from eggs or larvae requires some time. Nonetheless, after the first dose most treated cats were microscopically negative and on Day 51 15/16 cats did not shed L1 after the second treatment. The cats positive on Day 49 or 51 were, however, negative in the previous and following fecal examinations, and by Day 63 all treated cats were negative at the Baermann test.

Some cats of Group 2 and Group 3 presented respiratory signs after treatment (Table 1). This feature is consistent with previous data registered in experimental (Traversa et al., 2022) and natural (Traversa et al., 2019b) studies, in which study animals showed a temporary worsening of their clinical conditions and respiratory distress, likely due to an inflammatory response to the death of adult *T. brevior* caused by the treatment. Moreover, a few treated cats occasionally presented mild respiratory signs, i.e. deepened respiratory sound and sniffing, until the end of the study. These signs might represent residues from previous lung damage or ongoing repair mechanisms but might also be attributed to other causes like newly acquired mild viral infections of the upper airways. Furthermore, this could most likely be due to the pathogenic potential of *T. brevior* in kittens and young cats, which can suffer of long-term damages even when appropriate parasiticide is administered (Crisi et al., 2015).

Accordingly, the adverse events detected in two cats were most likely in response to worms dying after the administration of Profender® and Felpreva® and not caused by the products themselves. Therefore, the treatment was well tolerated in all animals, and this confirms the safety data already obtained in naturally infected animals treated with Profender® (Diakou et al., 2019).

5. Conclusion

In conclusion, the present results obtained in laboratory conditions further support the efficacy and safety of emodepside contained in spot-on formulations for the treatment of cat troglstrongylosis, as previously demonstrated in naturally infected cats (Di Cesare et al., 2015; Traversa et al., 2019b). Therefore, the use of emodepside contained in Profender® and Felpreva® is a reliable option for treating cats infected with *T. brevior*. The life-threatening potential of troglstrongylosis acquired vertically from the queen to the litter calls for further studies aiming at evaluating the efficacy of emodepside contained in Profender® and/or Felpreva® in interrupting the development of *T. brevior* from the infectious L3 to adulthood in kittens and young cats. For instance, Profender® has been already proven efficacious in preventing the vertical transmission of *Toxocara cati* from queen to kittens (Wolken et al., 2009; Böhm et al., 2015).

Funding

This study has been funded by Bayer Animal Health GmbH (now part of Elanco Animal Health). Profender® and Felpreva® are produced, licensed, and marketed by Vetoquinol in Europe. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Ethical approval and consent to participate

The study complied with (i) the Directive 2010/63/EU of the European Parliament and of the Council of 22nd September 2010 on the protection of the animals used for scientific purposes, (ii) the German animal protection act and (iii) the German welfare regulations for laboratory animals. Experiments conducted in cats were approved by the Ethics Commission of the Animal Care and Use Committee of the German Lower Saxony State Office for Consumer Protection and Food Safety (Niedersächsisches Landesamt fuer Verbraucherschutz und

Lebensmittelsicherheit - LAVES) under reference number 33.9-42502-04-19/3235. Snail breeding and infection was approved by the Italian Ministry of Health (DGSAF 0019336-P 15/07/2019), and by the Interinstitutional Ethical Committee for Animal Experimentation (CEISA - Prot. N. 03/2019).

CRedit author statement

Claudia Boehm, Hannah Ringeisen, Katrin Blazejak and Matthias Pollmeier have been involved in the design of the study, writing of study protocol, and monitoring of the study. Donato Traversa, Simone Morelli, Angela Di Cesare carried out the preparation of infection material (*Troglostrongylus brevior*). Christina Strube, Katharina Raue, Katrin Bisterfeld conducted the study, infected the cats and evaluated the study results. Christina Strube and Donato Traversa evaluated the study results and prepared the study report. Donato Traversa and Norbert Mencke wrote the manuscript. All authors read and approved the final manuscript.

Declaration of competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Claudia Boehm, Matthias Pollmeier and Hannah Ringeisen have been employees of Bayer Animal Health GmbH, an Elanco Animal Health Company during the study. Katrin Blazejak and Norbert Mencke are employees of Vetoquinol. Vetoquinol is the owner of all rights to Felpreva®. Donato Traversa, Simone Morelli, Angela Di Cesare, Christina Strube, Katharina Raue and Katrin Bisterfeld declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful to Drs Mariasole Colombo and Raffaella Iorio, and Mr Roberto Bartolini for their support in the snail breeding.

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