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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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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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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 troglostrongylosis 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 troglostrongylosis.

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 ~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 ~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× *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 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®.

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
	6577	70	Deepened respiratory sounds
2 (Profender®)	6344	−1	Slightly underweight
	6345	29	Coughing
		35	Deepened respiratory sound
	6554	35; 55; 70	Deepened respiratory sound
	6585	28	Coughing
3 (Felpreva®)	6550	−1	Small trace of blood on the anus after taking body temperature
	6433	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.

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 troglostrongylosis 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 troglostrongylosis 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. brevior*. 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 troglostrongylosis (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. brevior* 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. brevior* 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. brevior*, in a purposed field trial. These studies proved that two administrations 2 weeks apart (in combination with praziquantel in Profender®) were efficacious against *T. brevior* 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 troglostrongylosis.

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. brevior*. 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. brevior* (Crisi et al., 2018; Knaus et al., 2020). The study data submitted gained marketing authorization of Felpreva® for the treatment of infection with *T. brevior*.

Although it is hard to explain why some untreated cats were negative for adult *T. brevior* 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. brevior*. In endemic areas troglostrongylosis is a frequent disease in cats aged $\leq$ 6 months, whilst it is rarer in older cats up to 2 years of age and seldom or not diagnosed in cats aged $\geq$ 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. brevior*, with a frequent spontaneous death and elimination of worms.

Despite the negativity for adults of *T. brevior* 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. brevior* 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

deceasing 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 troglostrongylosis, 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 troglostrongylosis 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).

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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 (Niedersaechisches 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.

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References

Ambrosi, M., 1995. Parassitologia Zootecnica. Edagricole, Bologna, Italy, pp. 26–27.

Beugnet, F., 2021. NexGard® Combo (esafoxolaner, eprinomectin, praziquantel), a new endectoparasiticide spot-on formulation for cats. Parasite 28, E1. https://doi.org/10.1051/parasite/2021013.

Böhm, C., Petry, G., Schaper, R., Wolken, S., Strube, C., 2015. Prevention of lactogenic *Toxocara cati* infections in kittens by application of an emodepside/praziquantel spot-on (Profender®) to the pregnant queen. Parasitol. Res. 114 (Suppl. 1), S175–S184. https://doi.org/10.1007/s00436-015-4523-y.

Cavalera, M.A., Iatta, R., Colella, V., Dantas-Torres, F., Corsaro, A., Brianti, E., Otranto, D., 2018. *Troglostrongylus brevior*: a feline lungworm of paediatric concern. Vet. Parasitol. 253, 8–11. https://doi.org/10.1016/j.vetpar.2018.02.017.

Cavalera, M.A., Colella, V., Napoli, E., Arfuso, F., Panarese, R., Brianti, E., Otranto, D., 2019. Shedding of feline lungworm larvae and their infectivity to snail intermediate hosts after anthelmintic treatment. Int. J. Parasitol. 49, 449–453. https://doi.org/10.1016/j.ijpara.2018.12.008.

Crisi, P.E., Traversa, D., Di Cesare, A., Luciani, A., Civitella, C., Santori, D., Boari, A., 2015. Irreversible pulmonary hypertension associated with *Troglostrongylus brevior* infection in a kitten. Res. Vet. Sci. 102, 223–227. https://doi.org/10.1016/j.rvsc.2015.08.019.

Crisi, P.E., Aste, G., Traversa, D., Di Cesare, A., Febo, E., Vignoli, M., Santori, D., et al., 2017. Single and mixed feline lungworm infections: clinical, radiographic and therapeutic features of 26 cases (2013–2015). J. Feline Med. Surg. 19, 1017–1029. https://doi.org/10.1177/1098612X16670563.

Crisi, P.E., Di Cesare, A., Boari, A., 2018. Feline troglostrongylosis: current epizootiology, clinical features, and therapeutic options. Front. Vet. Sci. 5, 126. https://doi.org/10.3389/fvets.2018.00126.

Di Cesare, A., Crisi, P.E., Di Giulio, E., Veronesi, F., Frangipane di Regalbono, A., Talone, T., Traversa, D., 2013. Larval development of the feline lungworm *Aelurostrongylus abstrusus* in *Helix aspersa*. Parasitol. Res. 112, 3101–3108. https://doi.org/10.1007/s00436-013-3484-2.

Di Cesare, A., Frangipane di Regalbono, A., Tessarin, C., Seghetti, M., Iorio, R., Simonato, G., Traversa, D., 2014. Mixed infection by *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* in kittens from the same litter in Italy. Parasitol. Res. 113, 613–618. https://doi.org/10.1007/s00436-013-3690-y.

Di Cesare, A., Veronesi, F., Frangipane di Regalbono, A., Iorio, R., Traversa, D., 2015. Novel molecular assay for simultaneous identification of neglected lungworms and heartworms affecting cats. J. Clin. Microbiol. 53, 3009–3013. https://doi.org/10.1128/JCM.00901-15.

Diakou, A., Di Cesare, A., Barros, L.A., Morelli, S., Halos, L., Beugnet, F., Traversa, D., 2015. Occurrence of *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* in domestic cats in Greece. Parasit. Vectors 8, 590. https://doi.org/10.1186/s13071-015-1200-z.

Diakou, A., Sofroniou, D., Di Cesare, A., Kokkinos, P., Traversa, D., 2017. Occurrence and zoonotic potential of endoparasites in cats of Cyprus and a new distribution area for *Troglostrongylus brevior*. Parasitol. Res. 116, 3429–3435. https://doi.org/10.1007/s00436-017-5651-3.

Diakou, A., Morelli, S., Dimzas, D., Di Cesare, A., Capelli, G., Parrinello, C., et al., 2019. Efficacy of a moxidectin/imidacloprid spot-on formulation (Advocate®) for the treatment of *Troglostrongylus brevior* in naturally infected cats in a field study in Greece. Parasit. Vectors 12, 519. https://doi.org/10.1186/s13071-019-3760-9.

EMA, 2000a. VICH GL7 Efficacy of Anthelmintics: General Requirements, November 2000. https://www.ema.europa.eu/en/vich-gl7-efficacy-anthelmintics-general-requirements. (Accessed 15 January 2022).

EMA, 2000b. VICH GL9 Good Clinical Practices, July 2000. https://www.ema.europa.eu/en/vich-gl9-good-clinical-practices. (Accessed 15 January 2022).

EMA, 2001. VICH GL20 Efficacy of Anthelmintics: Specific Recommendations for Felines, June 2001. https://www.ema.europa.eu/en/vich-gl20-efficacy-anthelmintics-specific-recommendations-felines. (Accessed 15 January 2022).

Giannelli, A., Brianti, E., Varcasia, A., Colella, V., Tamponi, C., Di Paola, G., et al., 2015. Efficacy of Broadline® spot-on against *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* lungworms in naturally infected cats from Italy. Vet. Parasitol. 209, 273–277. https://doi.org/10.1016/j.vetpar.2015.02.037.

Giannelli, A., Capelli, G., Joachim, A., Hinney, B., Losson, B., Kirkova, Z., et al., 2017. Lungworms and gastrointestinal parasites of domestic cats: a European perspective. Int. J. Parasitol. 47, 517–528. https://doi.org/10.1016/j.ijpara.2017.02.003.

Knaus, M., Visser, M., Mayr, S., Rehbein, S., 2020. Efficacy of a topical combination of eprinomectin, praziquantel, fipronil and (S)-methoprene against developing and adult *Troglostrongylus brevior* lungworms (Nematoda, Crenosomatidae) in cats. Vet. Parasitol. 4, 100032. https://doi.org/10.1016/j.vpoa.2020.100032.

Morelli, S., Traversa, D., Colombo, M., Raue, K., Strube, C., Pollmeier, M., Di Cesare, A., 2020. The effect of the hibernation on the larval development of *Troglostrongylus brevior* in the land snail *Cornu aspersum*. Vet. Parasitol. 282, 109123. https://doi.org/10.1016/j.vetpar.2020.109123.

Morelli, S., Diakou, A., Colombo, M., Di Cesare, A., Barlaam, A., Dimzas, D., Traversa, D., 2021. Cat respiratory nematodes: current knowledge, novel data and warranted studies on clinical features, treatment and control. Pathogens 10, 454. https://doi.org/10.3390/pathogens10040454.

Pennisi, M.G., Hartmann, K., Addie, D.D., Boucraut-Baralon, C., Egberink, H., Frymus, T., et al., 2015. Lungworm disease in cats: ABCD guidelines on prevention and management. J. Feline Med. Surg. 17, 626–636. https://doi.org/10.1177/1098612X15588455.

Salant, H., Yasur-Landau, D., Rojas, A., Otranto, D., Mazuz, M.L., Baneth, G., 2020. *Troglostrongylus brevior* is the dominant lungworm infecting feral cats in Jerusalem. Parasitol. Res. 119, 3443–3450. https://doi.org/10.1007/s00436-020-06852-8.

Traversa, D., Salda, L.D., Diakou, A., Sforzato, C., Romanucci, M., Frangipane di Regalbono, A., et al., 2018. Fatal patent troglostrongylosis in a litter of kittens. J. Parasitol. 104, 418–423. https://doi.org/10.1645/17-172.

Traversa, D., Morelli, S., Cassini, R., Crisi, P.E., Russi, I., Grillotti, E., et al., 2019a. Occurrence of canine and feline extra-intestinal nematodes in key endemic regions of Italy. Acta Trop. 193, 227–235. https://doi.org/10.1016/j.actatropica.2019.03.009.

Traversa, D., Veronesi, F., Danesi, P., Morelli, S., Crisi, P.E., Morganti, G., et al., 2019b. Pilot study evaluating the efficacy of a topical formulation containing emodepside and praziquantel in the treatment of natural feline troglostrongylosis. Parasites Vectors 12, 97. https://doi.org/10.1186/s13071-019-3361-7.

Traversa, D., Morelli, S., Di Cesare, A., Diakou, A., 2021. Felid cardiopulmonary nematodes: dilemmas solved and new questions posed. Pathogens 10, 30. https://doi.org/10.3390/pathogens10010030.

Traversa, D., Raue, K., Ringeisen, H., Blazejak, K., Bisterfeld, K., Di Cesare, A., et al., 2022. Efficacy of a spot-on combination containing 10% w/v imidacloprid and 1% w/v moxidectin for the treatment of troglostrongylosis in experimentally infected cats. Parasit. Vectors 15, 66. https://doi.org/10.1186/s13071-022-05185-y.

Wolken, S., Schaper, R., Mencke, N., Kraemer, F., Schnieder, T., 2009. Treatment and prevention of vertical transmission of *Toxocara cati* in cats with an emodepside/praziquantel spot-on formulation. Parasitol. Res. 105 (Suppl. 1), S75–S81. https://doi.org/10.1007/s00436-009-1498-6.

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Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing tigolaner, emodepside and praziquantel, in treating cats with mixed infection with intestinal nematodes, cestodes and/or lungworms

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ABSTRACT

This paper describes a multicentric field study which has evaluated the safety and efficacy of a novel spot on formulation containing emodepside 2.04% w/v, praziquantel 8.14% w/v and tigolaner 9.79% w/v (Felpreva®, Vetoquinol) when administered at the intended commercial dose of 0.15 ml/kg body weight to privately owned cats infected with major intestinal nematodes (*Toxocara cati*, *Toxascaris leonina*, *Ancylostoma tubaeforme*, *Uncinaria stenocephala*) and/or cestodes (*Dipylidium caninum*, *Taenia taeniaeformis*) and/or lungworms (*Aelurostrongylus abstrusus*, *Troglostrongylus brevior*). A total of 219 cats from 26 veterinary clinics located in Albania, Greece, Hungary, Italy and Portugal were included in the study. Feces from the cats were examined on a single occasion between Study Day −7 and Day 0 (baseline) and post-treatment (i) twice between Day 7 and Day 14 (± 2) (for intestinal helminths) or (ii) twice between Day 21 (± 2) and Day 28 (± 2) (for lungworms). Cats were allocated into two groups at a ratio of 2:1 (Felpreva®: Profender®, i.e. a commercial control product containing emodepside and praziquantel). Cats infected with intestinal helminths were treated once on Day 0 (i) with Felpreva® (Group 1) or (ii) with Profender® (Group 2). Animals infected with lungworms received a second treatment with Profender® on Day 14 (± 2) regardless of group allocation. Faecal egg or larval count reduction for Felpreva® was 97.47% for intestinal nematodes and 96.80% for lungworms. No cats infected with cestodes at baseline resulted positive after treatment with Felpreva®. However, the low number of cats ($n = 10$) did not allow for a statistical analysis to be performed. Non-inferiority of Felpreva® compared to Profender® was statistically demonstrated for all target intestinal and respiratory parasites. No adverse events nor application site reactions were observed. These results show that the new topical combination product Felpreva® is highly safe and efficacious in treating infections caused by major species of feline intestinal nematodes, cestodes and lungworms under field conditions.

1. Introduction

Domestic cats may be infected with several endoparasites, intestinal nematodes and cestodes (tapeworms) and respiratory nematodes being the most important and distributed across Europe (Giannelli et al., 2017; Genchi et al., 2021).

The most important intestinal nematodes affecting domestic cats in Europe and elsewhere are the roundworm *Toxocara cati* and the

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hookworm *Ancylostoma tubaeforme*, followed by *Toxascaris leonina* and *Uncinaria stenocephala* (Nagamori et al., 2020; Traversa, 2012; Beugnet et al., 2014; Diakou et al., 2017). These parasites have a relevant clinical impact, especially for kittens and young animals, although cats of all ages may be infected. Cats harboring *T. cati* may be either subclinically infected or, most often, suffer of a catarrhal enteritis with vomitus, constipation or diarrhoea, delayed developmental rates, up to gut perforation and migration of adult worms in the abdominal cavity (Hendrix, 1995; Traversa, 2012). Hookworms cause enteritis, diarrhoeic feces, blood loss, anaemia, reduced weight, and may be deadly even when small numbers live in the small intestine of cats (Kalkofen, 1987; Traversa, 2012).

The most distributed felid intestinal cestodes are the flea-borne *Dipylidium caninum* and the taeniid *Taenia taeniaeformis*. Although the infections are most commonly subclinical, these worms may cause emesis, reduced growth rates, abdominal pain and discomforting anal pruritus (Bowman et al., 2002; Beugnet & Halos, 2015; Traversa & Venco, 2019).

Nematodes inhabiting the airways of cats have recently become a priority in feline medicine and *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* are now considered primary parasites of domestic cats from European countries (Morelli et al., 2021a; Traversa et al., 2021). These parasites cause respiratory diseases of varying severity, characterized by coughing, dyspnoea, sneezing, wheezing and general distress, which can be life-threatening especially in the case of troglostrongylosis in kittens and young animals (Morelli et al., 2021a).

Some of these helminths pose a sanitary risk for humans due to their zoonotic potential. This is the case of *T. cati* and *D. caninum*, which cause human *larva migrans* syndromes and intestinal diseases, respectively. It is worthy of note that these zoonotic diseases are of high sanitary relevance for children and immunocompromised subjects (Fisher, 2003; Deplazes et al., 2011; Hogan & Schwenk, 2019; Morelli et al., 2021b).

Geographical distribution and epidemiological patterns of these helminths depend on a plethora of intrinsic and extrinsic drivers, e.g. animal age, habitat, lifestyle, predatory behavior and availability of intermediate and/or paratenic hosts. Importantly, most of them have overlapping sources of transmission, e.g. *via* the ingestion of water or soil contaminated with infective stages or ingestion of small preys acting as intermediate or paratenic hosts (Morelli et al., 2021b). Hence, mixed infections with intestinal nematodes and cestodes, and lungworms are frequent in populations of domestic cats (Capári et al., 2013; Beugnet et al., 2014; Little et al., 2015).

Therefore, there is a high merit in controlling endoparasites of domestic cats towards mitigating clinical impact, minimizing environmental contamination, and reducing the risk of exposure and transmission to other animals and human beings. Broad spectrum medications have the high potential to treat animals infected with multiple parasites at the same time and, when they contain ecto- and endoparasiticides, are powerful to treat cats infested and/or infected with multiple external and internal parasites.

A new topical endectoparasiticide (Felpreva®, Vetoquinol) for cats combines tigolaner, emodepside and praziquantel. Tigolaner is an acaricide and insecticide belonging to the chemical class of bispyrazole. Emodepside and praziquantel, two anthelmintic molecules, demonstrated efficacy against nematodes and cestodes respectively, i.e. the cyclic depsipeptide emodepside and the pyrazino-isoquinoline praziquantel (Altreuther et al., 2005; Böhm et al., 2015; Traversa et al., 2019). This paper describes a field study that evaluated the therapeutic efficacy and safety of Felpreva® against infections caused by intestinal nematodes and/or cestodes and/or by lungworms in domestic cats from different countries in Europe.

2. Materials and methods

2.1. Study design

The study was carried out between February and July 2019. This was a blinded parallel group, controlled, randomized, multicenter and multi-regional field study conducted in accordance with Veterinary International Conference on Harmonization Guidelines (VICH Guideline 7: "Efficacy of anthelmintics: General requirements" and VICH Guideline 9, "Guideline on Good Clinical Practice" (EMA, 2000a, b)). The aim was to evaluate the efficacy and safety of a topical solution containing emodepside 2.04% w/v, praziquantel 8.14% w/v and tigolaner 9.79% w/v (Felpreva®, Vetoquinol) against mixed infection with intestinal and respiratory helminths, when administered once at the intended commercial dose of 0.15 ml/kg body weight (BW), corresponding to a minimum of 3 mg/kg BW, 12 mg/kg BW and 14.4 mg/kg BW for emodepside, praziquantel and tigolaner, respectively. Felpreva® was evaluated for non-inferiority in comparison to a control product authorized in the EU market for the treatment of the target species of this study, i.e. a spot-on containing emodepside 2.1% w/v and praziquantel 8.6% w/v (Profender®, Vetoquinol). Concurrent infestations with ectoparasites (fleas, ticks, and mites) were also documented during the study (Cvejić et al., 2022).

2.2. Study sites, cat population and target parasites

According to VICH Guideline 7, field studies are to be conducted in different geographical and climatic regions, thus at least two countries in different climatic regions were selected. A total of 27 client-owned cats attending veterinary clinics in Albania ($n = 4$), Greece ($n = 2$), Hungary ($n = 8$) Italy ($n = 5$), and Portugal ($n = 8$) were recruited in the efficacy study.

The target parasite species were intestinal nematodes (*T. cati*, *T. leonina*, *A. tubaeforme* and *U. stenocephala*) and/or cestodes (*D. caninum* and *T. taeniaeformis*) and lungworms (*A. abstrusus* and *T. brevior*).

2.3. Study scheme

Cats were screened for the study between Day −7 and Day 0, and animals meeting all the following inclusion criteria, but none of the exclusion criteria were enrolled.

Inclusion criteria were as follows: (i) minimum 1.0 kg BW and 10 weeks-old on Day 0; (ii) positive for intestinal nematodes and/or cestodes and/or lungworms at the qualitative or quantitative copromicroscopy between Day −7 and Day 0; (iii) physical examination on Day 0; (iv) compliance and written consent of the owner or authorized representative; and (v) manageability of the cats. The following animals were excluded from the study: (i) females intended for breeding during the study until 4 months following the last dosing; (ii) pregnant or lactating queens; (iii) cats with history of apparent reactions to the IVP (Investigational Veterinary Product, Felpreva® Vetoquinol) and/or CP (Control Product, Profender® Vetoquinol); (iv) history of deworming at a dosage and regimen with proven efficacy against targeted parasites within 12 weeks prior to Day 0; and (v) pre-existing medical and/or surgical condition except for routine surgical procedures.

Cats were randomized in accordance with a Random Treatment Allocation Plan using a block design at a 2:1 ratio (Felpreva®: Profender®). When more than one cat was present in a household, all cats meeting the inclusion criteria were included in the study and allocated to the same treatment group.

Faecal samples were collected from each study cat and examined in a centralized laboratory on a single occasion between Day −7 and Day 0 (pre-study screening, baseline), twice between Day 7 and Day 14 (± 2) (post-treatment evaluation in case of infection with intestinal nematodes and/or cestodes) or twice between Day 21 (± 2) and Day 28 (± 2) (post-treatment evaluation in case of infection with *A. abstrusus* and/or *T. brevior*).

Cats infected with intestinal nematodes and/or cesrtodes were treated once on Day 0 with IVP (Group 1) or CP (Group 2). Animals which were infected with *A. abstrusus* and/or *T. brevior* received a first treatment with IVP on Day 0 and a second treatment with the control product Profender® on Day 14 (± 2) independent of the group allocation.

2.4. Parasitological procedures

Faecal samples were refrigerated until shipment to the laboratory, where they were macroscopically examined and then subjected to microscopic conventional quantitative centrifugation-flotation by FLOTAC® and Baermann techniques, in order to detect parasitic eggs/oocysts or larvae. The FLOTAC techniques use the FLOTAC apparatus and are based on the centrifugal flotation of a faecal sample suspension and subsequent translation of the apical portion of the floating suspension (Cringoli et al., 2010). Egg counts were performed as follows: 10 g of feces (or less if not available) were diluted with 90 ml of tap water (if less than 10 g were available, the corresponding dilution ratio of 1:10 has been used). The faecal suspension was homogenized and filtered, and the 11 ml were placed in a conic tube and centrifugated at 170× *g*. Thereafter, the supernatant was discarded, and zinc sulphate solution (specific gravity 1350) was added to the sediment to reach a 11 ml volume and then thoroughly homogenized. The two flotation chambers of the FLOTAC® have been then filled with the suspension, the device was centrifuged at 120× *g* and then examined under an optical microscope.

The qualitative Baermann examination was performed using 5–10 g of feces placed in a cheesecloth that was closed to form a pouch. The latter was placed in a Baermann funnel filled with water, closed at the bottom and kept at room temperature. After 12–24 h, 15 ml of faecal fluid were drawn off the bottom into a tube and centrifuged at 600× *g* for 5 min. Thereafter, the supernatant was discarded, the sediment was placed onto a glass slide and examined under the optical microscope.

The quantitative Baermann examination was performed using 1 g of faces placed on a double-layered gauze, settled into a Baermann funnel filled with 50 ml of tap water. After 24 h, the solution was poured into a tube and centrifuged at 600× *g*. The supernatant was removed, and the sediment was examined under an optical microscope. Larvae were morphologically identified and counted to assess the number of L1 per gram of feces (LPG). Any parasitic element retrieved at FLOTAC® or Baermann examination was morphologically and morphometrically examined (Sloss et al., 1994; Traversa & Di Cesare, 2016). Additional PCR analysis of faecal samples positive for lungworms at the Baermann assay were carried out for a definitive discrimination between *A. abstrusus* and *T. brevior* (Di Cesare et al., 2015a).

2.5. Physical examinations

Study animals underwent a physical examination prior to inclusion on Day 0 and for study completion on Day 14 (± 2) (cats infected with intestinal nematodes and/or cestodes) or Day 28 (± 2) (cats infected with lungworms). The possible occurrence of adverse events (AEs) was monitored at planned physical examinations and during the whole study.

2.6. Efficacy assessment and criteria

Efficacy criteria were analyzed within each of the three subgroups of animals infected with target parasites, i.e. intestinal nematodes, cestodes or lungworms. First primary criterion was non-inferiority of Felpreva® compared to Profender® in percent reduction of faecal egg/larval count (FEC/FLC) between Day −7 to Day 0 (baseline) and Day 7 to Day 14 (± 2) or Day 21 (± 2) to Day 28 (± 2) (post-treatment) for intestinal nematodes and cestodes or lungworms, respectively. In the presence of non-inferiority, reduction was further analyzed by comparison of the two groups based on a threshold of two FECs/FLCs in the post-baseline counts.

A 5% level of significance ($P < 0.05$ for two-sided tests) was used to assess statistical differences (i.e. one-sided significance level of 2.5%).

2.6.1. Intestinal nematodes

The efficacy endpoint was the FECs at baseline (one sample collected between Day −7 and Day 0) compared to the post-treatment values (Day 7 to Day 14 (± 2)) per each individual cat. Percent efficacy was obtained for each identified species/genus and for each study group separately. If one or both post-treatment FEC values were above zero, the higher value was used for efficacy calculation. The FEC of each species/genus was transformed to the natural logarithm of (count + 1) for the calculation of geometric means. The percent efficacy was calculated using formula 100 × [(B – T)/B], where B is the geometric mean of the study group on pre-treatment sample and T is the geometric mean of the study group on Day 7 to Day 14 (± 2).

In particular, the FEC reduction was assessed separately for *T. cati* and *A. tubaeforme* with the objective to have a > 90% mean geometric FEC reduction between baseline and Day 7 to Day 14 (± 2). A comparison of Group 1 and Group 2 was performed by non-inferiority analysis with a 0.15 (15%) threshold. In the presence of non-inferiority, the post-baseline FEC values of IVP and CP groups were compared using a non-inferiority threshold of two FECs. In this case a two-sided 95% confidence interval (CI) was computed on the IVP Group – CP Group difference of log-faecal egg counts with an upper limit (one-sided 97.5% confidence limit) ≤ 0.69 ($\log_2$).

2.6.2. Intestinal cestodes

The efficacy endpoint was the FEC at baseline compared to FECs between Day 7 and Day 14 (± 2) per each individual cat. An additional efficacy parameter was presence/absence of cestode eggs and/or proglottids at baseline compared to Day 7 to Day 14 (± 2). The low number of animals positive for cestodes did not allow for a statistical evaluation.

2.6.3. Respiratory nematodes

The reduction of FLC from the baseline (Day −7 to Day 0) to the post-treatment values (Day 21 (± 2) to Day 28 (± 2)) was the primary efficacy criterion for lungworms (*A. abstrusus* or *T. brevior*). If one or both post treatment FLCs were above zero, the higher value was used for calculation of efficacy. The percent efficacy was calculated as described in *Section* 2.6.1 for intestinal nematodes.

3. Results

3.1. Study cats

Overall, 930 cats were screened, and, of them, 219 animals scored positive at qualitative or quantitative copromicroscopy and were recruited in the study. Of these 219 cats (i.e. the Intention-to-Treat (ITT) population), 144 were treated with Felpreva® and 75 with Profender®. A total of 201 animals completed the study, i.e. 133 in Group 1 and 68 in Group 2. The Per-Protocol (PP) feline population, i.e. the total of cats with no major deviations from the protocol, consisted of 195 cats, i.e. 127 and 68 cats treated with Felpreva® or Profender®, respectively.

3.2. Baseline infections

Parasites found in the ITT and PP populations are reported in Table 1. In total, 105 cats in Group 1 and 56 cats in Group 2 were infected with intestinal nematodes. Of them, 142 (92 in Group 1 and 50 in Group 2) were infected with the roundworm *T. cati*, 27 (18 in Group 1 and 9 in Group 2, respectively) with the hookworm *A. tubaeforme* and 10 with the minor species *T. leonina* or *U. stenocephala* (6 in Group 1 and 4 in Group 2).

Ten cats scored positive for cestodes. Proglottids were detected in 5 animals (3 in Group1 and 2 in Group 2), while egg packets of *D. caninum* (3 cats) and eggs of *T. taeniaeformis* (2 cats) were detected in 5 other faecal samples (4 in Group 1 and 1 in Group 2).

PCRs for lungworms conducted on 33 samples belonging to the ITT population showed that 10 cats harbored *T. brevior* (6 in Group 1 and 4 in Group 2) and 22 cats harbored *A. abstrusus* (13 in Group 1 and 9 in Group 2), while in one case the PCR did not confirm the microscopy result. Of those 32 cats in the PP population, 18 and 14 cats were included in Group 1 and Group 2, respectively. With regard to mixed infections, 17 animals

Table 1
Results of the screening (Day −7 to Day 0) faecal examination of 219 cats included in the present study. Concomitant infestations by fleas and mites are also reported

	ITT population			PP population		
	Total	Felpreva® (Group 1)	Profender® (Group 2)	Total	Felpreva® (Group 1)	Profender® (Group 2)
Included [a]	219	144	75	195	127	68
Intestinal nematodes [b]	166	109	57	161	105	56
Toxocara cati	147	96	51	142	92	50
Toxascaris leonina	7	4	3	7	4	3
Ancylostoma tubaeforme	27	18	9	27	18	9
Uncinaria stenocephala	4	3	1	4	3	1
Cestodes [c]	10	7	3	9	6	3
Taenia taeniaeformis	2	1	1	2	1	1
Dipylidium caninum	3	3	0	3	3	0
Lungworms [d]	33	19	14	32	18	14
Aelurostrongylus abstrusus	22	13	9	21	12	9
Troglostrongylus brevior	10	6	4	10	6	4
Ectoparasites (total)	70	44	26	58	37	21
Fleas	59	37	22	48	31	17
Ear mites	4	2	2	4	2	2
Fleas and ear mites	7	5	2	6	4	2

Abbreviations: ITT, Intention-To-Treat population; PP, Per-Protocol population.
[a] Out of 930 screened cats.
[b] 17 cats harboured mixed infections with *Toxocara cati*, *Ancylosoma tubaeforme*, *Toxascaris leonina*, and/or *Uncinaria stenocephala*.
[c] Total sum based on cats with positive FEC and cats with presence of proglottids, species were only determined in 5 animals with FEC > 0 at baseline.
[d] In one sample the lungworm species could not be confirmed by PCR.

Table 2
Percent reduction of log-transformed faecal egg counts (FEC) or faecal larval counts (FLC) for intestinal nematodes and lungworms in the Per-Protocol (PP) population of the present study

Species	Statistic	T1: IVP (A)	T2: CP (B)	Difference[a] B - A
Intestinal nematodes		*N* = 105	*N* = 56	
Toxocara cati	*n*	92	50	
	Mean ± SD	96.37 ± 15.22	96.54 ± 12.55	0.17 ± 14.34
	95% CI	96.22–99.52	92.97–100.11	−4.813–5.15
	Min-Max	0.39–100	32.13–100	
	Median	100	100	
Ancylostoma tubaeforme	*n*	18	9	
	Mean ± SD	100 ± 0	100 ± 0	0 ± 0
	Min-Max	100–100	100–100	
	Median	100	100	
Others (*Toxascaris leonina*, *Uncinaria stenocephala*)	*n*	6	4	
	Mean ± SD	100 ± 0	100 ± 0	0 ± 0
	Min-Max	100–100	100–100	
	Median	100	100	
Any nematodes[b]	*n*	105	56	
	Mean ± SD	97.47 ± 12.04	97.95 ± 7.92	0.48 ± 10.07
	95% CI	95.33–99.61	95.82–100.07	−2.815–3.77
	Min-Max	25.22–100	62.62–100	
	Median	100	100	
Lungworms		*N* = 18	*N* = 14	
	n	18	14	
	Mean ± SD	96.80 ± 13.56	97.98 ± 7.545	1.18 ± 11.35
	95% CI	90.06–103.55	93.63–102.34	−7.08–9.44
	Min-Max	42.47–100	71.77–100	
	Median	100	100	
Aelurostrongylus abstrusus	*n*	12	10	
	Mean ± SD	95.21 ± 16.61	97.18 ± 8.93	1.97 ± 13.69
	95% CI	84.65–105.76	90.79–103.56	−10.26–14.20
	Min-Max	42.47–100	71.77–100	
	Median	100	100	
Troglostrongylus brevior	*n*	6	4	
	Mean ± SD	100 ± 0	100 ± 0	0 ± 0
	95% CI	–	–	–
	Min-Max	100–100	100–100	
	Median	100		

Abbreviations: IVP, Investigational Veterinary Product, Felpreva® Vetoquinol; CP, Control Product, Profender® Vetoquinol; *n*, number of cats infected per parasite species; *N*, total number of cats per group; SD, standard deviation; CI, confidence interval; Min, minimum; Max, maximum.
[a] 95% confidence interval from ANOVA.
[b] If a cat was infected with more than one species, sum of FEC was evaluated. In 17 cats more than one intestinal nematode species was found (10 in the Felpreva® group and 7 in the Profender® group).

(10.3%) were positive for at least two different intestinal nematodes, i.e. 13 cats had a mixed infection with *T. cati* and *A. tubaeforme*, two with *T. leonina* and *T. cati*, one with *T. leonina* and *A. tubaeforme* and one with *T. cati*, *A. tubaeforme*, *T. leonina* and *U. stenocephala*. Nine cats (4.1%) harbored intestinal nematodes, lungworms and/or cestodes. Seventy cats (31.9%) were concurrently infested with fleas (87.1%) and/or ear mites (12.8%) but none of them with ticks.

3.3. Efficacy and safety evaluations

The analysis of efficacy was based on PP population. All cats that received at least one dose of Felpreva® or Profender® were included in the assessment of Safety Population (SP).

3.3.1. Intestinal nematodes

Reduction of FEC on Day 7 to Day 14 (± 2) for all intestinal nematodes was 97.47% and 97.95% in Felpreva® and Profender® groups, respectively. Reduction of *T. cati* FEC was 96.37% and 96.54% in the Felpreva®- and Profender®-treated cats, respectively, while all post-treatment counts were reduced to 0 for *A. tubaeforme*, *T. leonina* and *U. stenocephala* for all cats belonging to the two study groups, i.e. 100% FEC reduction in both groups (Table 2). Non-inferiority of Felpreva® compared to Profender® was shown when analysing all intestinal nematodes and *T. cati* alone, i.e. 3.77% and 5.15% upper bound of the 95% CI respectively. The non-inferiority of Felpreva® shown based on the mean geometric FEC reduction was confirmed by analysis of the non-inferiority threshold of two FECs.

3.3.2. Cestodes

None of the cats with the presence of proglottids at baseline had proglottids present on post-treatment evaluations in both study groups. Analogously, no cestode packet/eggs were detected in the feces of cats which scored positive at the baseline copromicroscopy. The low number of cestode-positive cats did not allow a statistical evaluation.

3.3.3. Lungworms

Post-treatment FLC reduction was 96.80% and 97.98% in the Felpreva® and Profender® group, respectively, considering both lungworm species. Reduction calculated separately was 95.21 *vs* 97.18% in the Felpreva® and Profender® groups for *A. abstrusus*, and 100% for *T. brevior* in both groups (Table 2).

Non-inferiority of Felpreva® compared to Profender® was demonstrated by 9.44% (both lungworms) and 14.20% (*A. abstrusus* only) upper bound of the 95% CI. As for the intestinal nematodes, the non-inferiority of Felpreva® was confirmed by analysing the non-inferiority threshold of two FLCs.

3.3.4. Safety

No AEs occurred during the trial; therefore, no assessment was done. No application site reactions were observed after treatment in any of the study cats.

4. Discussion

It was the aim of the present study to evaluate the efficacy and safety of a novel topical broad spectrum parasiticide containing emodepside 2.04 w/v, praziquantel 8.14 w/v and tigolaner 9.79 w/v (Felpreva®, Vetoquinol) when administered at minimum dosages to cats infected with major intestinal nematodes and/or cestodes and/or lungworms.

With regard to intestinal nematodes, the efficacy of the product was well above the requested threshold of 90% including all species found. Although the number of cats infected with minor roundworms (i.e. *T. leonina*) and hookworms (*U. stenocephala*) did not allow a separate statistical evaluation, this did not prevent confirming the efficacy of Felpreva®. In fact, the percent reduction was 96.37% for *T. cati*, up to 100% for the other species together. Moreover, non-inferiority of Felpreva®-treated with the Profender®-treated group was proven in all these efficacy analyses.

Similarly, a statistical analysis of efficacy data was not conducted for cestodes, due to the low number of cats positive for proglottids and/or eggs. However, all cats positive for cestode elements at baseline scored negative after treatment. Considering that praziquantel is a cestocide with a well-established effectiveness and there are no data available of loss of activity, there is no reason to exclude that the IVP is efficacious against target species *D. caninum* and *T. taenieformis*. The results obtained for respiratory metastrongyloids, both from the reduction of FLC and additional PCRs conducted to distinguish between *A. abstrusus* and *T. brevior*, showed that Felpreva® is effective against both lungworm species. The efficacy against *T. brevior* in experimentally infected cats has also recently been published (Traversa et al., 2022).

Importantly, the efficacy evaluation was supported by the results of the non-inferiority results obtained with a control commercial product licensed for most of these indications. In particular, many data are published on the efficacy of emodepside and praziquantel in treating infections caused by the target species (Altreuther et al., 2005; Reinemeyer et al., 2005; Di Cesare et al., 2015b; Lee et al., 2019; Traversa et al., 2019; Crisi et al., 2020).

Mixed infections are common in cats especially because of the overlapping transmission patterns of many parasites. Indeed, cats living outdoors or allowed to free-roam are more prone to be infected with different endo- and/or ectoparasites at the same time. This however does not imply that cats living indoors are out of parasite risk, because they may acquire infections or infestations from different sources, like raw meat, preying on indoor small animals and dirty soil (Morelli, 2021).

The results of the present multicentric studies confirm that client-owned feline populations of Europe are often at risk of infection by intestinal and/or respiratory helminths and, in many cases, of ectoparasites. Data recently originated from other surveys (Giannelli et al., 2017; Genchi et al., 2021) further corroborate this scenario, and ultimately highlight that broad spectrum parasiticides are crucial to control endo- and ectoparasites under certain epidemiological scenarios where cats are at risk of mixed infections/infestations. Accordingly, emodepside and praziquantel contained in the here evaluated Felpreva® are efficacious for the treatment of most common feline intestinal and respiratory nematodes, and intestinal tapeworms, respectively. It is also worthy of note that the here evaluated Felpreva® contained tigolaner, an ectoparasiticide efficacious against ectoparasites which may concurrently infest cats harboring helminths (Cvejić et al., 2022).

5. Conclusion

In conclusion, this new combination proved to be highly safe and efficacious in the treatment of infections caused by intestinal nematodes, cestodes and lungworms in privately owned cats under field conditions. Hence, the new Felpreva® product will be extremely useful under the epidemiological setting where client-owned feline populations are at risk to be parasitized by these helminths and/or common ectoparasites (Cvejić et al., 2022).

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Ethical approval

A clinical field study confirming the efficacy and safety of a veterinary medicinal product is required to obtain the marketing authorization according to Directive (2004)/28/EC and 2009/9/EC amending 2001/82/EC in Europe. Cat owners agreed to the participation of their animals in

the study prior to enrolment and initiation of treatment, in terms of treatment, collection procedures, and visits to veterinary practices at the required time.

CRediT author statement

Gabriele Petry, Hannah Ringeisen and Hannah Hamburg have been involved in the design of the study, writing of study protocol, and monitoring of the study. Dejan Cvejić, Klaus Hellmann, Donato Traversa, Simone Morelli, Angela Di Cesare and Anastasia Diakou conducted the multicenter study with the veterinary clinics involved, evaluating and reporting the study results. Róbert Farkas conducted and reported the parasite diagnosis. Donato Traversa, Norbert Mencke and Dejan Cvejić 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: Gabriele Petry, Hannah Ringeisen, and Hannah Hamburg have conducted and funded the study and are employees of Bayer Animal Health GmbH, an Elanco Animal Health company. Dejan Cvejić and Klaus Hellmann are employees of Klifovet GmbH Munich, Germany. Norbert Mencke is an employee of Vetoquinol, Paris, France. Vetoquinol is the owner of the product Felpreva reported within this study. Róbert Farkas, Donato Traversa, Simone Morelli, Angela Di Cesare and Anastasia Diakou declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Altreuther, G., Buch, J., Charles, S.D., Davis, W.L., Krieger, K.J., Radeloff, I., 2005. Field evaluation of the efficacy and safety of emodepside/praziquantel spot-on solution against naturally acquired nematode and cestode infections in domestic cats. Parasitology 97, S58–S64. https://doi.org/10.1007/s00436-005-1445-0.

Beugnet, F., Bourdeau, P., Chalvet-Monfray, K., Cozma, V., Farkas, R., Guillot, J., et al., 2014. Parasites of domestic owned cats in Europe: co-infestations and risk factors. Parasit. Vectors 7, 291. https://doi.org/10.1186/1756-3305-7-291.

Beugnet, F., Halos, L. (Eds.), 2015. Parasitoses and vector-borne diseases of cats. Merial, Lyon, France.

Böhm, C., Wolken, S., Schnyder, M., Basso, W., Deplazes, P., Di Cesare, A., et al., 2015. Efficacy of emodepside/praziquantel spot-on (Profender®) against adult *Aelurostrongylus abstrusus* nematodes in experimentally infected cats. Parasitol. Res. 114, S155–S164. https://doi.org/10.1007/s00436-015-4521-0.

Bowman, D.D., Hendrix, C.M., Lindsay, D.S., Barr, S.C., 2002. Feline clinical parasitology. Iowa State University. A Blackwell Science Company, USA.

Capári, B., Hamel, D., Visser, M., Winter, R., Pfister, K., Rehbein, S., 2013. Parasitic infections of domestic cats, *Felis catus*, in western Hungary. Vet. Parasitol. 192, 33–42. https://doi.org/10.1016/j.vetpar.2012.11.011.

Cringoli, G., Rinaldi, L., Maurelli, M.P., Utzinger, J., 2010. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat. Protoc. 5, 503–515. https://doi.org/10.1038/nprot.2009.235.

Crisi, P.E., Di Cesare, A., Traversa, D., Vignoli, M., Morelli, S., Di Tommaso, M., et al., 2020. Controlled field study evaluating the clinical efficacy of a topical formulation containing emodepside and praziquantel in the treatment of natural cat aelurostrongylosis. Vet. Rec. 187, e34. https://doi.org/10.1136/vr.105528.

Cvejić, D., Hellmann, K., Petry, G., Ringeisen, H., Hamburg, H., Farkas, R., Blazejak, K., Mencke, N., 2022. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing emodepside, praziquantel and tigolaner, in treating cats naturally infested with fleas and/or ticks. Curr. Res. Parasitol. Vector Borne Dis. 2, 100098.

Diakou, A., Di Cesare, A., Accettura, P.M., Barros, L., Iorio, R., Paoletti, B., et al., 2017. Intestinal parasites and vector-borne pathogens in stray and free-roaming cats living in continental and insular Greece. PLoS Negl. Trop. Dis. 11, e0005335. https://doi.org/10.1371/journal.pntd.0005335.

Di Cesare, A., Veronesi, F., Frangipane di Regalbono, A., Iorio, R., Traversa, D., 2015a. Novel molecular assay for simultaneous identification of neglected lungworms and heartworms affecting cats. J. Clin. Microbiol. 53, 3009–3013. https://doi.org/10.1128/JCM.00901-15.

Di Cesare, A., Iorio, R., Crisi, P., Paoletti, B., Di Costanzo, R., Dimitri, C.F., Traversa, D., 2015b. Treatment of *Troglostrongylus brevior* (Metastrongyloidea, Crenosomatidae) in mixed lungworm infections using spot-on emodepside. J. Feline Med. Surg. 17, 181–185. https://doi.org/10.1177/1098612X14533552.

Deplazes, P., van Knapen, F., Schweiger, A., Overgaauw, P.A., 2011. Role of pet dogs and cats in the transmission of helminthic zoonoses in Europe, with a focus on echinococcosis and toxocarosis. Vet. Parasitol. 182, 41–53. https://doi.org/10.1016/j.vetpar.2011.07.014.

EMA, 2000a. VICH GL7 Efficacy of anthelmintics: General requirements, November 2000. https://www.ema.europa.eu/en/vich-gl7-efficacy-anthelmintics-general-requirements. (Accessed 15 January 2022).

EMA, 2000b. VICH GL9 Good Clinical Practices, July 2000. https://www.ema.europa.eu/en/vich-gl9-good-clinical-practices. (Accessed 15 January 2022).

Fisher, M., 2003. *Toxocara cati*: an underestimated zoonotic agent. Trends Parasitol. 19, 167–170. https://doi.org/10.1016/s1471-4922(03)00027-8.

Genchi, M., Vismarra, A., Zanet, S., Morelli, S., Galuppi, R., Cringoli, G., et al., 2021. Prevalence and risk factors associated with cat parasites in Italy: a multicenter study. Parasit. Vectors 14, 475. https://doi.org/10.1186/s13071-021-04981-2.

Giannelli, A., Capelli, G., Joachim, A., Hinney, B., Losson, B., Kirkova, Z., et al., 2017. Lungworms and gastrointestinal parasites of domestic cats: a European perspective. Int. J. Parasitol. 47, 517–528. https://doi.org/10.1016/j.ijpara.2017.02.003.

Hendrix, C.M., 1995. Helminth infections of the feline small and large intestine: diagnosis and treatment. Vet. Med. 90, 456–476.

Hogan, C.A., Schwenk, H., 2019. *Dipylidium caninum* infection. N. Engl. J. Med. 380, e39. https://doi.org/10.1056/NEJMicm1813985.

Kalkofen, U.P., 1987. Hookworms of dogs and cats. Vet. Clin. North Am. Small. Anim. Pract. 17, 1341–1354. https://doi.org/10.1016/s0195-5616(87)50005-5.

Lee, S.H., Ock, Y., Choi, D., Kwak, D., 2019. Gastrointestinal parasite infection in cats in Daegu, Republic of Korea, and efficacy of treatment using topical emodepside/praziquantel formulation. Korean J. Parasitol. 57, 243–248. https://doi.org/10.3347/kjp.2019.57.3.243.

Little, S., Adolph, C., Downie, K., Snider, T., Reichard, M., 2015. High prevalence of covert infection with gastrointestinal helminths in cats. J. Am. Anim. Hosp. Assoc. 51, 359–364. https://doi.org/10.5326/JAAHA-MS-6221.

Morelli, S., 2021. The indoor cat: do the endoparasites knock at the door?. In: Proceedings of Conference "XXXI Congresso SoIPa & 2021 ESDA EVENT", Teramo, 16-19 June 2021. SoIPA.

Morelli, S., Diakou, A., Colombo, M., Di Cesare, A., Barlaam, A., Dimzas, D., Traversa, D., 2021a. Cat respiratory nematodes: current knowledge, novel data and warranted studies on clinical features, treatment and control. Pathogens 10, 454. https://doi.org/10.3390/pathogens10040454.

Morelli, S., Diakou, A., Di Cesare, A., Colombo, M., Traversa, D., 2021b. Canine and feline parasitology: analogies, differences, and relevance for human health. Clin. Microbiol. Rev. 34. https://doi.org/10.1128/CMR.00266-20 e00266-20.

Nagamori, Y., Payton, M.E., Looper, E., Apple, H., Johnson, E.M., 2020. Retrospective survey of parasitism identified in feces of client-owned cats in North America from 2007 through 2018. Vet. Parasitol. 277, 109008. https://doi.org/10.1016/j.vetpar.2019.109008.

Reinemeyer, C.R., Charles, S.D., Buch, J., Settje, T., Altreuther, G., Cruthers, L., et al., 2005. Evaluation of the efficacy of emodepside plus praziquantel topical solution against ascarid infections (*Toxocara cati* or *Toxascaris leonina*) in cats. Parasitol. Res. 97, S41–S50. https://doi.org/10.1007/s00436-005-1443-2.

Sloss, M.W., Kemp, R.L., Zajac, A.M., 1994. Fecal examination: dogs and cats. In: Veterinary Clinical Parasitology, 6th ed. Iowa State University Press, Ames, USA.

Traversa, D., 2012. Pet roundworms and hookworms: a continuing need for global worming. Parasit. Vectors 5, 91. https://doi.org/10.1186/1756-3305-5-91.

Traversa, D., Di Cesare, A., 2016. Diagnosis and management of lungworm infections in cats: cornerstones, dilemmas and new avenues. J. Feline Med. Surg. 18, 7–20. https://doi.org/10.1177/1098612X15623113.

Traversa, D., Venco, L., 2019. Parassitologia clinica del cane e del gatto. Le Point Veterinaire Italie, Milano, Italy.

Traversa, D., Morelli, S., Di Cesare, A., Strube, C., Raue, K., Bisterfeld, K., Boehm, C., et al., 2022. Efficacy of a spot-on combination containing tigolaner, emodepside, & praziquantel (Felpreva®) in comparison to the spot-on combination of emodepside and praziquantel (Profender®) in experimentally infected cats with *Troglostrongylus brevior*. Curr. Res. Parasitol. Vector Borne Dis. 2 (In press, this issue).

Traversa, D., Veronesi, F., Danesi, P., Morelli, S., Crisi, P.E., Morganti, G., et al., 2019. Pilot study evaluating the efficacy of a topical formulation containing emodepside and praziquantel in the treatment of natural feline troglostrongylosis. Parasit. Vectors 12, 97. https://doi.org/10.1186/s13071-019-3361-7.

Traversa, D., Morelli, S., Di Cesare, A., Diakou, A., 2021. Felid cardiopulmonary nematodes: dilemmas solved and new questions posed. Pathogens 10, 30. https://doi.org/10.3390/pathogens10010030.

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Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing emodepside, praziquantel and tigolaner, in treating cats naturally infested with fleas and/or ticks

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ABSTRACT

The present field study evaluated the safety and 3-month preventive efficacy of a novel spot-on endectocide containing emodepside 2.04% w/v, praziquantel 8.14% w/v and tigolaner 9.79% w/v (Felpreva®, Vetoquinol) when administered at the intended commercial dose of 0.15 ml/kg body weight to privately owned cats infested by fleas (*Ctenocephalides felis*) and/or ticks (*Ixodes ricinus*, *Ixodes hexagonus*, *Rhipicephalus* spp.). The efficacy of Felpreva® to reduce the clinical signs associated with flea allergy dermatitis was also evaluated. A total of 326 cats, i.e. 120 and 206 infested by ticks and fleas respectively, from 16 different sites located in Hungary and Portugal were included on Day 0 and allocated in two Groups at a ratio of 2:1 (T1:T2). Cats of T1 were treated with Felpreva®, while cats of T2 were dosed with a commercial Control Product (Bravecto®, MSD Animal Health) licensed for the same indications. Of the 120 tick-infested cats, 79 and 41 were treated with Felpreva® and Bravecto® respectively, while of the 206 flea-infested cats, 139 were treated with Felpreva® and 67 with Bravecto®. Cats were physically examined on Days 7, 28, 56, 75 and 90; when present, fleas and ticks were counted and collected. Efficacy evaluation was based on the mean percent reduction of live parasite counts for each of five visits *versus* the pre-treatment count. Percent reductions of live flea and tick counts over all post-baseline periods were 99.74% (T1) *versus* 98.56% (T2) and 97.50% (T1) *versus* 98.65% (T2), respectively. Non-inferiority for the Felpreva® compared with the Bravecto® treated group was statistically demonstrated for both fleas and ticks. Three adverse events were observed and considered unlikely related to the treatment. These results show that the new topical combination product Felpreva® is safe and highly efficacious in treating flea and tick infections in cats for at least three months (90 days) with a single administration. In 16 cats that were identified with flea allergy dermatitis, the clinical signs of flea allergy dermatitis improved following treatment in both groups.

1. Introduction

Fleas and ticks are common ectoparasites of cats in many countries (Pennisi et al., 2015; Lefkaditis et al., 2016; Tulloch et al., 2017; Geurden et al., 2018; Abdullah et al., 2019). These arthropods cause direct damages (e.g. blood deprivation, skin lesions, tick paralysis, flea-allergic dermatitis) and transmit vector-borne diseases (VBDs) of veterinary and public health interest (Hill et al., 2006; Morelli, 2021).

Fleas are the predominant ectoparasites of domestic cats, which can be infested at high rates with the cat flea *Ctenocephalides felis*, followed by *Ctenocephalides canis* (the dog flea) and *Pulex irritans* (the human flea) (Farkas et al., 2009; Knaus et al., 2014; Persichetti et al., 2016). Flea allergy dermatitis is one of the most important dermatological conditions in small animal veterinary medicine. Fleas may transmit different pathogens, i.e. the zoonotic tapeworm *Dipylidium caninum*, and bacteria of the genera *Bartonella*, *Mycoplasma* and *Rickettsia* (Hill et al., 2006; Farkas

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et al., 2009). Ticks are usually considered less frequent in cats than fleas, though feline infestations are not uncommon and there is evidence of a global increased prevalence of tick infestations in cats (Tulloch et al., 2017; Little et al., 2018). Cats living in Europe may harbour several species of ticks, *Rhipicephalus sanguineus* (*sensu lato*), *Ixodes ricinus*, *Ixodes hexagonus* and *Dermacentor reticulatus* being the most common (Ogden et al., 2000; Tulloch et al., 2017; Geurden et al., 2018). Ticks transmit relevant pathogens to cats, such as *Hepatozoon* spp., *Cytauxzoon* spp., *Ehrlichia* spp., *Anaplasma* spp. and *Borrelia* spp. (Little, 2010; Barker et al., 2019; Morelli et al., 2021). Many flea- and tick-borne pathogens have a recognized zoonotic potential (Kegler et al., 2018; Barker et al., 2019; Tørnqvist-Johnsen et al., 2020; Morelli et al., 2021).

The regular administration of appropriate ectoparasiticides is essential to control flea and tick infestation in cats and to reduce the risk of infection with the pathogens they may transmit. In recent years, different products containing isoxazolines have been licensed for use in cats infested with fleas and ticks (Geurden et al., 2017; Cavalleri et al., 2018a, b; Rohdich et al., 2018; Beugnet, 2021). Tigolaner is a newly developed molecule belonging to the chemical class of bispyrazoles and, though it is not an isoxazoline, it has the same efficacious mechanism of action against arthropods, i.e. it acts as antagonist of GABA-regulated chloride channels (International nonproprietary names for pharmaceutical substances: https://www.who.int/publications/i/item/who-emp-rht-tsn-2018-1).

The present study has investigated the efficacy and safety of a novel spot-on formulation containing tigolaner along with emodepside and praziquantel (Felpreva®, Vetoquinol) when administered to domestic cats naturally infested with ticks and/or fleas.

2. Materials and methods

2.1. Study design

This study was a controlled, randomized and blinded parallel group multicenter field study conducted in accordance with Veterinary International Conference on Harmonization Guidelines (VICH GL 9) (EMA, 2000) and to the EMEA/CVMP/EWP/005/2000-Rev.2 "Guidelines for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestations in dogs and cats" (EMEA, 2008).

The preventive efficacy and safety of a topical solution (Felpreva®) containing emodepside 2.04% w/v, praziquantel 8.14% w/v and tigolaner 9.79% w/v (Felpreva®, Vetoquinol) was evaluated in cats naturally infested with fleas and ticks, when administrated once at the intended commercial dose of 0.15 ml/kg body weight (BW), corresponding to a minimum of 3 mg/kg BW, 12 mg/kg BW and 14.4 mg/kg BW for emodepside, praziquantel and tigolaner respectively.

Felpreva® was evaluated for non-inferiority with a positive control product authorized for the target species in the EU market, i.e. a spot-on containing fluralaner (Bravecto®, MSD Animal Health).

2.2. Study sites, cat population and target parasites

As per guidelines two countries located in different geographical areas and with varying climatic conditions were selected. The study population consisted of client-owned cats presented at 16 veterinary practices equally located in Hungary and Portugal. The practices were selected in territories known for high prevalence of tick and/or flea infestation in companion animals. The target parasites were fleas (*C. felis*) and the common tick species (*I. ricinus*, *I. hexagonus*, *D. reticulatus* and *R. sanguineus* (*s.l.*)). Cat owners agreed to the participation of their animals in the study prior to enrolment and initiation of treatment, in terms of treatment, flea and/or tick count and collection procedures, and visits to veterinary practices at the required times.

2.3. Inclusion criteria

Cats were recruited before or at study Day 0 according to the following inclusion criteria: (i) cats living in households with a maximum of 3 cats and 2 dogs (maximum: 5 animals); (ii) cats with detected fleas and ticks ($\geq$ 5 viable fleas and $\geq$ 3 attached and viable ticks); and (iii) adequate physical examination on Day 0.

Moreover, cats: (i) showing both (i.e. $\geq$ 5 viable fleas and $\geq$ 3 attached and viable ticks) were randomized according to the randomization list for tick households; (ii) with tick infestation ($\geq$ 3 attached and viable ticks) but with less than 5 viable fleas (i.e. not meeting the inclusion criteria for flea infestation) were included and randomized as "tick patients"; (iii) with flea infestation ($\geq$ 5 viable fleas) but with less than 3 attached and viable ticks (i.e. not meeting the inclusion criteria for tick infestation) were included and randomized as "flea patients".

2.4. Exclusion criteria

The following animals were excluded from the study: (i) cats weighing less than 1.2 kg BW or less than 11 weeks-old on Day 0; (ii) females intended for breeding during the study until 4 months following the last dosing; (iii) queens known or suspected to be pregnant or lactating; (iv) cats with any history of apparent reactions to the Felpreva® and/or Bravecto® or any of their active compounds; (v) cats treated with an ectoparasiticide at a dosage and regimen known to provide efficacy against ticks and or fleas within the 12 weeks prior to Day 0; (vi) pre-existing medical and/or surgical condition except for routine surgical procedures.

2.5. Randomization

Cats were randomized per single household (flea or tick) according to a 2:1 ratio (Felpreva®: Bravecto®) in two groups, i.e. T1 (animals treated with Felpreva®) and T2 (animals treated with Bravecto®). All cats from the same household received the same treatment.

One cat per household was nominated as primary patient for efficacy and safety evaluations. In particular, if more than one cat in a household met the inclusion criteria, the cat with the highest number of fleas ($\geq$ 5 viable fleas) or ticks ($\geq$ 3 attached and viable ticks) was designated as the primary cat. All other cats in the same household were considered as supplementary patients and received safety evaluations.

Dogs living in the same household with cat(s) included into the study were treated with an adequate ectoparasiticide to eliminate flea infestation between animals.

2.6. Treatment

Cats infested with fleas and/or ticks were treated on Day 0 with Felpreva® (T1) or Bravecto® (T2) by the Dispenser in the clinic. Treatment dispensing was based on the body weights recorded on Day 0. Cats were dosed once with the appropriate pipette size of Felpreva® or Bravecto® to provide the recommended minimum dosage of 14.4 mg tigolaner + 3 mg emodepside + 12 mg praziquantel/kg body weight (Felpreva®) or following manufacturer's recommendations to deliver 40 mg fluralaner/kg body weight (Bravecto®). Both products Felpreva® and Bravecto® were administered topically directly on the skin of the cats. Application was done with cat standing and application on the cat's neck at the base of the skull, while the hair was divided with two fingers in this region until the skin was visible. The whole pipette volume was applied directly to the skin at one spot. Care was taken not to spill any product. The cat was restrained for about 1 min to allow the product to spread. No applied product got lost during administration.

2.7. Physical examinations and parasitological procedures

Cats enrolled in the study were subjected to a physical examination and body weighing on Day 0 (prior to inclusion), and post treatment at Day 7 (± 1), Day 28 (± 2), Day 56 (± 2), Day 75 (± 2) and Day 90 (± 2). The physical examination included an evaluation of clinical signs possibly related to flea allergy dermatitis and a visual inspection (thumb inspection and combing) for ectoparasites. The total body surface was combed with a flea comb provided. Each cat was combed for at least 10 min and the combing extended for at least another 5 min after the last flea was found. Tick assessment was carried out by thumb count, pushing the hair against its natural lap, thus skin and attached ticks are exposed, beginning at the head and systematically cover all areas of the animal.

A full body count was done for each study animal. Fleas and/or ticks eventually present on the animal were counted, categorized in viable/dead and attached/not attached (ticks), collected and appropriately stored.

A physical examination was performed on Day 0 (+ 2) and on Day 90 (± 2), and optionally on Day 7 (± 1), Day 28 (± 2), Day 56 (± 2) and Day 75 (± 2) for supplementary cats.

Adverse events (AEs) were evaluated at all physical examinations, and the application site was evaluated on Day 0 (prior to inclusion), and Day 7 (± 1) and Day 28 (± 2). Study completion was on Day 90 (± 2), or, in case of cat removal prior to Day 90 (± 2), on the day when the animal was removed from the study.

2.8. Efficacy assessment

The statistical unit was one cat per household nominated as primary patient for efficacy assessments. Efficacy criteria were separately assessed for non-inferiority by comparing post-baseline flea and tick counts with the control group.

Baseline comparability of treatment groups was assessed by means of descriptive tables on the following baseline information on Day 0: animal characteristics (breed, sex, age, hair type and body weight), animal husbandry, physical examinations for primary and supplementary cats separately and the parasite counts on Day 0 (live fleas and/or ticks) for primary cats only.

The primary efficacy criterion was the efficacy in terms of percent reduction for each visit (average of all visits) of the Felpreva®-treated group compared to the Bravecto®-treated group over the entire treatment period compared to baseline based on counts of live fleas and live and attached ticks. The secondary efficacy criterion was the efficacy of the Felpreva®-treated group compared to the Bravecto®-treated group for each separate visit compared to baseline, based on counts of live flea and live and attached ticks. This value was assessed as the percent reduction of flea and tick counts for each visit, separately.

As parasite counts in general show a strongly skewed distribution, a natural logarithmic transformation {ln (count + 1)} was applied to flea and tick counts and percentage reduction was calculated on transformed counts. Both, arithmetic and geometric mean of log-transformed counts were used for percentage reduction calculation.

Least squares means of percentage reduction over all post-baseline periods for the Felpreva®- and Bravecto®-treated groups were calculated from an analysis of variance with repeated measurements adjusted for baseline (main effect of treatment over all post-baseline periods). Considering the negative sign of reduction, non-inferiority was accepted, if the upper limit of the one-sided 97.5% confidence interval of the difference of μ_{IVP} - μ_{CP} was smaller than $\Delta = 15\%$. The 5% level of significance ($P < 0.05$ for two-sided tests) was used to assess statistical differences (corresponding to a one-sided significance level of 2.5%).

3. Results

3.1. Study cats

In total 529 cats were considered suitable for enrolment in the study. The Intention-to-Treat (ITT) population consisted of primary flea-infested ($n = 206$) and tick-infested ($n = 120$) cats, and 139 and 79 of them were treated with Felpreva® (T1) and 67 and 41 with Bravecto® (T2), respectively.

Serious deviations from study protocol occurred for three and one primary flea-infested and tick-infested cats, respectively, thus leading to their exclusion from the Per-Protocol (PP) population, i.e. the total of cats with no major deviations from the protocol and included in the analysis of efficacy criteria. Thus, the PP population consisted of 203 (137 Felpreva®-treated, 66 Bravecto®-treated) and 119 (79 Felpreva®-treated, 40 Bravecto®-treated) cats, respectively, for flea and tick efficacy analysis. Regarding supplementary animals, i.e. 137 for fleas and 66 for ticks, 94 and 39 were treated with Felpreva® and 43 and 27 with Bravecto®, respectively.

3.2. Baseline infestations

On Day 0, the mean number of live fleas found in study cats was 10.6 (minimum–maximum: 5–47) and 12.4 (minimum–maximum: 5–150) in the Felpreva® and Bravecto® group, respectively. In tick-infested cats, the mean numbers of live ticks and fleas were 3.7 (minimum–maximum: 3–7 for Felpreva® group and 3–6 for Bravecto® group) and 0.8 (minimum–maximum: 0–14 for Felpreva® group and 0–8 for Bravecto® group) for the Felpreva® and Bravecto® group, respectively. All fleas were identified as *C. felis*, while the most common tick retrieved was *I. ricinus*, followed by *D. reticulatus*, *R. sanguineus* (*s.l.*) and *I. hexagonus* (Table 1).

3.3. Efficacy and safety evaluations

The analysis of efficacy was based on primary cats PP population. A supportive efficacy analysis was obtained based on primary cats of the ITT population. All animals which received at least one dose of Felpreva® or Bravecto® were included in the assessment of Safety Population (SP), which corresponded to the ITT population. The analysis of safety was performed for primary and supplementary cats.

3.3.1. Primary efficacy

Percentage reduction of live flea and tick counts over all post-baseline periods was 99.74% and 98.56% (fleas) and 97.50% and 98.65% (ticks) in the Felpreva® and Bravecto® treatment groups,

Table 1
Flea and tick species found at baseline: Per Protocol Population

	Total	Felpreva® group	Bravecto® group
Ticks (*N*)[a]	119	79	40
Ixodes ricinus (*n*, %)	80 (67.2)	55 (69.6)	25 (62.5)
Ixodes hexagonus (*n*, %)	5 (4.2)	2 (2.5)	3 (7.5)
Rhipicephalus sanguineus (*s.l.*) (*n*, %)	35 (29.4)	24 (30.4)	11 (27.5)
Dermacentor reticulatus (*n*, %)	36 (30.3)	23 (29.1)	13 (32.5)
Other species (*n*, %)	4 (3.4)	3 (3.8)	1 (2.5)
Not identified (*n*, %)	1 (0.8)	0 (0)	1 (2.5)
Fleas (*N*)[a]	203	137	66
Ctenocephalides felis (*n*, %)	199 (98.0)	135 (98.5)	64 (97.0)

Abbreviations: *N*, number of animals; *n*, number of ticks/fleas.
[a] Some cats were infected by more than one tick species at baseline.

Table 2
Percentage reduction of flea and ticks counts over all post-baseline periods: Per Protocol Population

	Felpreva®	Bravecto®	Difference Bravecto® – Felpreva®	95% CI
Fleas				
No. of cats	137	66	–	–
Arithmetic mean ± SD	2.34 ± 0.46	2.34 ± 0.58	–	–
Geometric mean	9.39	9.40	–	–
Mean percent reduction over all post-baseline periods	−99.7387	−98.5651	−1.1736	−1.7558 to −0.5914
Ticks				
No. of cats	79	40	–	–
Arithmetic mean ± SD	1.52 ± 0.18	1.52 ± 0.20	–	–
Geometric mean	3.58	3.58	–	–
Mean percent reduction over all post-baseline periods	−97.5016	−98.6521	1.1505	0.2287–2.0724

Note: Data shown for Day 0 (+2).
Abbreviation: CI, confidence interval; SD, standard deviation.

respectively. Non-inferiority of Felpreva®-treated group compared to Bravecto®-treated group was shown by the 0.59% and 2.07% upper bound of the 95% confidence interval (CI) for fleas and ticks, respectively (Table 2).

3.3.2. Secondary efficacy

Percentage reduction of flea counts in the Felpreva® treatment group was 99.2% on Day 7, 99.8% on Day 28, 100% on Days 56 and 75, and 99.7% on Day 90. In the Bravecto® treatment group percentage reduction of flea counts was 99.0% on Day 7, 100% on Days 28 and 56, 99.2% on Day 75, and 98.5% on Day 90. Non-inferiority of the Felpreva® compared to the Bravecto® treatment group could be concluded for each study visit for the duration of 3 months (90 days) (data not shown).

Percentage reduction of tick counts in the Felpreva® group was 100% on Days 7, 28, 56 and 75, and 99.2% on Day 90. In the Bravecto® group, percentage reduction of tick counts was 100% on Days 7, 28 and 75, 99.1% on Day 56, and 98.1% on Day 90 (Table 3). Non-inferiority of the Felpreva® compared to the Bravecto® could be concluded for each study visit for the duration of 3 months (90 days). Flea allergy dermatitis (FAD) was assessed at study start in all cats (n = 529 primary as well as supplementary cats) based on pre-defined clinical signs (pruritus, crusts/scabs, papules, erythema, scaling and/or alopecia) all to be rated as being present (mild/moderate/severe) or absent.

Based on these criteria, overall 24 cats (4.5%) were diagnosed with FAD on day 0 (16 in the Felpreva® and 8 in the Bravecto® group). All these animals had no FAD sign at the study completion.

3.3.3. Efficacy versus single tick species

PP populations for each single tick species consisted of 23 and 13 (*D. reticulatus*), 55 and 25 (*I. ricinus*), and 24 and 11 (*R. sanguineus* (*s.l.*)) cats in the Felpreva® and Bravecto® group, respectively. The low number of cats infested with *I. hexagonus* (n = 5) prevented a statistical analysis.

Percentage reduction of *D. reticulatus* counts was 100% on all study days for the Felpreva® group and 97.3–100% from Day 7 (± 1) to Day 90 (± 2) for the Bravecto® group. Regarding *I. ricinus* counts, the percentage reduction in the Felpreva® group was constantly 100% on all study days except for Day 90 (± 2) (percentage reduction of 98.8%). The reduction in the Bravecto® group was 97–100% from Day 7 (± 1) to Day 90 (± 2).

Table 3
Mean percentage reduction of fleas and ticks counts in Felpreva® and Bravecto® groups

Day	7 (± 1)	28 (± 2)	56 (± 2)	75 (± 2)	90 (± 2)
Fleas					
Felpreva®	99.2	99.8	100	100	99.7
Bravecto®	99.0	100	100	99.2	98.5
Ticks					
Felpreva®	100	100	100	100	99.2
Bravecto®	100	100	99.1	100	98.1

The percentage reduction for *R. sanguineus* (*s.l.*) tick counts was 100% on all study days in the cats for both treatment groups (Table 4).

3.3.4. Safety

There was one serious adverse event (cat hit by car and died) observed in one of the Felpreva®-treated and three non-serious adverse events of the Bravecto®-treated cats. All four AEs were evaluated as unlikely related to the treatment.

4. Discussion

The present results show that the novel topical broad spectrum parasiticide containing emodepside 2.04% w/v, praziquantel 8.14% w/v and tigolaner 9.79% w/v (Felpreva®, Vetoquinol) is efficacious and safe when administered to cats infested with fleas or ticks at the minimum recommended dose. It could be confirmed that Felpreva® has a persistent efficacy over three months (90 days) after a single dose against live fleas and ticks, with a percent reduction of 99.7% and 99.2%, respectively. Non-inferiority with a commercial product already licensed for this indication was proven.

All fleas isolated from the study cats were identified as *C. felis*, i.e. the dominant flea species infesting cat populations in Europe (Gálvez et al., 2017). At the same time, efficacy data obtained for individual tick species regard the most important and spread species affecting felines in Europe (Claerebout et al., 2013; Geurden et al., 2017; Rohdich et al., 2018). In this view, the efficacy of Felpreva® against the three tick species (*I. ricinus*, *D. reticulatus* and *R. sanguineus* (*s.l.*)) affecting the vast majority of enrolled cats identified was remarkably high over a period of 90 days, i.e. constantly 100% with the sole exception of a percentage reduction of 98.8% for *I. ricinus* on Day 90 (± 2) (study completion).

The reliability of the present study was confirmed by data on infestation pressure for study cats. To assure that study cats were under infestation pressure during the whole study, the environmental challenge for ectoparasite infestations was descriptively evaluated based on other dogs and cats presented to the veterinary practices. These animals were

Table 4
Percentage reduction of different tick species counts in Felpreva® and Bravecto® groups

Day	7 (± 1)	28 (± 2)	56 (± 2)	75 (± 2)	90 (± 2)
Dermacentor reticulatus					
Felpreva®	100	100	100	100	100
Bravecto®	100	100	100	100	100
Ixodes ricinus					
Felpreva®	100	100	100	100	98.8
Bravecto®	100	100	98.6	100	97.0
Rhipicephalus sanguineus (*s.l.*)					
Felpreva®	100	100	100	100	100
Bravecto®	100	100	100	100	100

Note: Due to the low number of animals infested with *Ixodes hexagonus* (4.2%) no statistical evaluation was done.

infested with fleas and/or ticks, and/or required a control treatment for these ectoparasites.

Cats are constantly at risk to be (re-)infested with fleas and ticks from the environment. These pets thus require to be treated with medications which guarantee a persistent efficacy until the end of the treatment period, to control the direct clinical impact of these infestations and to minimize the clinical and epidemiological risk of vector-borne diseases. Fleas are traditionally considered as prevalent feline parasites whilst ticks in cats are erroneously of less concern. Nevertheless, recent data have proven that ticks are becoming a common pest of cat populations in Europe even where they are unexpected (Geurden et al., 2017; Rohdich et al., 2018; Wright, 2018; Buczek & Buczek, 2020). This recent information confirms a relatively new risk for cats represented by tick infestations and tick-borne pathogens. Thus, the high efficacy of Felpreva® against fleas and ticks is of importance not only for the direct pathogenic impact of these arthropods (e.g. anaemia, skin damages, allergic reactions) but also for the control of transmitted diseases. Although this was not investigated in the present study, it can be argued that Felpreva® has the potential to reduce the risk of pathogen transmission by fleas (e.g. *D. caninum*) to cats.

The use of broad-spectrum formulations containing an endo- and an ecto-parasiticide is particularly useful in cats living outdoors or allowed to free-roam, as they are at risk to acquire various internal and external parasites at the same time. In fact, large-scale studies have proven that cats of Europe are often simultaneously infected by internal cestodes and/or nematodes and/or external parasites (Beugnet et al., 2014; Giannelli et al., 2017; Genchi et al., 2021). Nonetheless, cats living indoors are also at risk of becoming infected by internal helminths *via* different routes (Morelli, 2021) and to be parasitized by arthropods. This is particularly true for fleas, which find in household indoor environments the best humidity and temperature parameters for their survival and reproduction (Dryden et al., 2011). Given that most pet cats are allowed to go outside (Foreman-Worsley et al., 2021) there is a frequent need to use broad spectrum parasiticides to control at the same time endo- and ecto-parasites affecting cats at risk of mixed infections and/or infestations. It is thus worthy of note that emodepside and praziquantel contained in the evaluated Felpreva® are efficacious against common intestinal nematodes and tapeworms, and lungworms (Altreuther et al., 2005; Reinemeyer et al., 2005; Di Cesare et al., 2015; Lee et al., 2019; Traversa et al., 2019; Crisi et al., 2020). The efficacy of Felpreva® against gastrointestinal nematodes and cestodes as well as lungworms was investigated, and efficacy shown by an equivalent multicenter field study (Cvejić et al., 2022).

The long efficacy duration against arthropods is an important feature of Felpreva®. Pets receiving a longer duration product are in general protected against fleas and ticks for more months per year compared to animals which receive formulations to be dosed monthly (Lavan et al., 2018, 2020, 2021). Possible gaps in terms of subsequent parasiticide administrations limit the time protection provided against ectoparasites, and the gap between administrations leaves the cat unprotected against fleas and ticks. This is of importance in terms of owner compliance as a recent survey has shown that cat owners have a common high level of preference of long-lasting formulations efficacious against fleas and ticks (Lavan et al., 2021). Such a high adherence to the use of long-lasting medications is probably due also to inferior number of administrations scheduled per year. As stress for pet cats (and probably for owners themselves) is a trigger for reducing the number of visits to the vets (Volk et al., 2011), a product assuring three months of protection against ticks and fleas after a single dose implies the advantage that owners are required to dose their cats once instead than three times in the same time interval.

5. Conclusion

The present results show that the new spot-on formulation Felpreva® containing tigolaner (plus emodepside and praziquantel) is efficacious and safe against natural flea and tick infestations in cats. A quick and persistent efficacy of ectoparasiticides is of utmost relevance under those field circumstances where cats are at risk to be (re-)infested by arthropods and, at the same time, are exposed to vector-borne pathogens. The duration of Felpreva® was proven to provide up to three months protection following a single dose. Such an approach allows a safe, efficacious, and long-lasting fleas and ticks control for cats.

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Ethical approval and consent to participate

A clinical field study confirming the efficacy and safety of a veterinary medicinal product is required to obtain the marketing authorization according to Directive (2004)/28/EC and 2009/9/EC amending 2001/82/EC in Europe. Cat owners agreed to the participation of their animals in the study prior to enrolment and initiation of treatment, in terms of treatment, flea and/or tick count and collection procedures, and visits to veterinary practices at the required times.

CRediT author statement

Gabriele Petry, Hannah Ringeisen, and Hannah Hamburg have been involved in the design of the study, writing of study protocol, and monitoring of the study. Dejan Cvejić and Klaus Hellmann conducted the multicenter study with the veterinary clinics involved, evaluating and reporting the study results. Róbert Farkas conducted and reported the parasite diagnosis and Katrin Blazejak and Norbert Mencke wrote the manuscript in conjunction with Dejan Cvejić. 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: Gabriele Petry, Hannah Ringeisen, and Hannah Hamburg have conducted and funded the study and are employees of Bayer Animal Health GmbH, an Elanco Animal Health company. Dejan Cvejić and Klaus Hellmann are employees of Klifovet GmbH Munich, Germany. Katrin Blazejak and Norbert Mencke are employees of Vetoquinol, Paris, France. Vetoquinol is the owner of the product Felpreva reported within this study. Róbert Farkas declares no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abdullah, S., Helps, C., Tasker, S., Newbury, H., Wall, R., 2019. Pathogens in fleas collected from cats and dogs: distribution and prevalence in the UK. Parasit. Vectors 6, 71. https://doi.org/10.1186/s13071-019-3326-x.

Altreuther, G., Buch, J., Charles, S.D., Davis, W.L., Krieger, K.J., Radeloff, I., 2005. Field evaluation of the efficacy and safety of emodepside/praziquantel spot-on solution against naturally acquired nematode and cestode infections in domestic cats. Parasitology 97, S58–S64. https://doi.org/10.1007/s00436-005-1445-0.

Barker, E.N., 2019. Update on feline hemoplasmosis. Vet. Clin. North. Am. Small Anim. Pract. 49, 733–743. https://doi.org/10.1016/j.cvsm.2019.02.009.

Beugnet, F., 2021. NexGard® Combo (esafoxolaner, eprinomectin, praziquantel), a new endectoparasiticide spot-on formulation for cats. Parasite 28, E1. https://doi.org/10.1051/parasite/2021013.

Beugnet, F., Bourdeau, P., Chalvet-Monfray, K., Cozma, V., Farkas, R., Guillot, J., et al., 2014. Parasites of domestic owned cats in Europe: co-infestations and risk factors. Parasit. Vectors 7, 291. https://doi.org/10.1186/1756-3305-7-291.

Buczek, A., Buczek, W., 2020. Importation of ticks on companion animals and the risk of spread of tick-borne diseases to non-endemic regions in Europe. Animals 11, 6. https://doi.org/10.3390/ani11010006.

Cavalleri, D., Murphy, M., Seewald, W., Nanchen, S., 2018a. A randomized, controlled field study to assess the efficacy and safety of lotilaner (Credelio™) in controlling ticks in client-owned cats in Europe. Parasites Vectors 11, 411. https://doi.org/10.1186/s13071-018-2967-5.

Cavalleri, D., Murphy, M., Seewald, W., Nanchen, S., 2018b. A randomized, controlled field study to assess the efficacy and safety of lotilaner (Credelio™) in controlling fleas in client-owned cats in Europe. Parasites Vectors 11, 410. https://doi.org/10.1186/s13071-018-2971-9.

Claerebout, E., Losson, B., Cochez, C., Casaert, S., Dalemans, A.C., De Cat, A., et al., 2013. Ticks and associated pathogens collected from dogs and cats in Belgium. Parasit. Vectors 6, 183. https://doi.org/10.1186/1756-3305-6-183.

Crisi, P.E., Di Cesare, A., Traversa, D., Vignoli, M., Morelli, S., Di Tommaso, M., et al., 2020. Controlled field study evaluating the clinical efficacy of a topical formulation containing emodepside and praziquantel in the treatment of natural cat aelurostrongylosis. Vet. Rec. 187, e34. https://doi.org/10.1136/vr.105528.

Di Cesare, A., Iorio, R., Crisi, P., Paoletti, B., Di Costanzo, R., Dimitri, C.F., Traversa, D., 2015. Treatment of *Troglostrongylus brevior* (Metastrongyloidea, Crenosomatidae) in mixed lungworm infections using spot-on emodepside. J. Feline Med. Surg. 17, 181–185. https://doi.org/10.1177/1098612X14533552.

Cvejić, D., Mencke, N., Petry, G., Ringeisen, H., Hamburg, H., Hellmann, K., Traversa, D., Morelli, S., Di Cesare, A., Diakou, A., Farkas, R., 2022. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing tigolaner, emodepside and praziquantel, in treating cats with mixed infection with intestinal nematodes, cestodes and/or lungworms. Curr. Res. Parasitol. Vector Borne Dis. 2, 100098.

Dryden, M.W., Carithers, D., Murray, M.J., 2011. Flea control: real homes, real problems, real answers, real lessons: fleas in a flash! Compend. Contin. Educ. Vet. 33, E5.

EMA, 2000. VICH GL9 Good Clinical Practices, July 2000. https://www.ema.europa.eu/en/vich-gl9-good-clinical-practices. (Accessed 15 December 2021).

EMEA, 2008. EMEA/CVMP/EWP/005/2000-Rev.2: Guideline for the Testing and Evaluation of the Efficacy of Antiparasitic Substances for the Treatment and Prevention of Tick and Flea Infestation in Dogs and Cats, 1 June 2008. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-testing-evaluation-efficacy-antiparasitic-substances-treatment-prevention-tick-flea_en.pdf. (Accessed 15 December 2021).

Farkas, R., Gyurkovszky, M., Solymosi, N., Beugnet, F., 2009. Prevalence of flea infestation in dogs and cats in Hungary combined with a survey of owner awareness. Med. Vet. Entomol. 23, 187–194. https://doi.org/10.1111/j.1365-2915.2009.00798.x.

Foreman-Worsley, R., Finka, L.R., Ward, S.J., Farnworth, M.J., 2021. Indoors or outdoors? An international exploration of owner demographics and decision making associated with lifestyle of pet cats. Animals 11, 253. https://doi.org/10.3390/ani11020253.

Gálvez, R., Musella, V., Descalzo, M.A., Montoya, A., Checa, R., Marino, V., et al., 2017. Modelling the current distribution and predicted spread of the flea species *Ctenocephalides felis* infesting outdoor dogs in Spain. Parasit. Vectors 10, 428. https://doi.org/10.1186/s13071-017-2357-4.

Genchi, M., Vismarra, A., Zanet, S., Morelli, S., Galuppi, R., Cringoli, G., et al., 2021. Prevalence and risk factors associated with cat parasites in Italy: a multicenter study. Parasit. Vectors 14, 475. https://doi.org/10.1186/s13071-021-04981-2.

Geurden, T., Becskei, C., Farkas, R., Lin, D., Rugg, D., 2017. Efficacy and safety of a new spot-on formulation of selamectin plus sarolaner in the treatment of naturally occurring flea and tick infestations in cats presented as veterinary patients in Europe. Vet. Parasitol. 238 (Suppl. 1), S12–S17. https://doi.org/10.1016/j.vetpar.2017.03.008.

Geurden, T., Becskei, C., Six, R.H., Maeder, S., Latrofa, M.S., Otranto, D., Farkas, R., 2018. Detection of tick-borne pathogens in ticks from dogs and cats in different European countries. Ticks Tick Borne Dis 9, 1431–1436. https://doi.org/10.1016/j.ttbdis.2018.06.013.

Giannelli, A., Capelli, G., Joachim, A., Hinney, B., Losson, B., Kirkova, Z., et al., 2017. Lungworms and gastrointestinal parasites of domestic cats: a European perspective. Int. J. Parasitol. 47, 517–528. https://doi.org/10.1016/j.ijpara.2017.02.003.

Hill, P.B., Lo, A., Eden, C.A., Huntley, S., Morey, V., Ramsey, S., et al., 2006. Survey of the prevalence, diagnosis and treatment of dermatological conditions in small animals in general practice. Vet. Rec. 158, 533–539. https://doi.org/10.1136/vr.158.16.533.

Kegler, K., Nufer, U., Alic, A., Posthaus, H., Olias, P., Basso, W., 2018. Fatal infection with emerging apicomplexan parasite *Hepatozoon silvestris* in a domestic cat. Parasit. Vectors 11, 428. https://doi.org/10.1186/s13071-018-2992-4.

Knaus, M., Rapti, D., Shukullari, E., Kusi, I., Postoli, R., Xhaxhiu, D., et al., 2014. Characterisation of ecto- and endoparasites in domestic cats from Tirana, Albania. Parasitol. Res. 113, 3361–3371. https://doi.org/10.1007/s00436-014-3999-1.

Lavan, R., Armstrong, R., Burgio, F., Tunceli, K., 2018. Duration of annual canine flea and tick protection provided by dog owners in Spain. Parasit. Vectors 11, 458. https://doi.org/10.1186/s13071-018-3043-x.

Lavan, R., Armstrong, R., Normile, D., Vaala, W., 2020. Adherence to veterinary recommendations for ectoparasiticides purchased by cat owners in the USA. Parasit. Vectors 13, 541. https://doi.org/10.1186/s13071-020-04415-5.

Lavan, R.P., Armstrong, R., Newbury, H., Normile, D., Hubinois, C., 2021. Flea and tick treatment satisfaction, preference, and adherence reported by cat owners in the US, UK, or France who treated their cats with trandayermal fluralaner. Open Vet. J. 11, 458–467. https://doi.org/10.5455/OVJ.2021.v11.i3.19.

Lee, S.H., Ock, Y., Choi, D., Kwak, D., 2019. Gastrointestinal parasite infection in cats in Daegu, Republic of Korea, and efficacy of treatment using topical emodepside/praziquantel formulation. Korean J. Parasitol. 57, 243–248. https://doi.org/10.3347/kjp.2019.57.3.243.

Lefkaditis, M.A., Athanasiou, L.V., Ionică, A.M., Koukeri, S.E., Panorias, A., Eleftheriadis, T.G., Boutsini, S., 2016. Ectoparasite infestations of urban stray dogs in Greece and their zoonotic potential. Trop. Biomed. 33, 226–230.

Little, S.E., 2010. Ehrlichiosis and anaplasmosis in dogs and cats. Vet. Clin. North Am. Small. Anim. Pract. 40, 1121–1140. https://doi.org/10.1016/j.cvsm.2010.07.004.

Little, S.E., Barrett, A.W., Nagamori, Y., Herrin, B.H., Normile, D., Heaney, K., Armstrong, R., 2018. Ticks from cats in the United States: patterns of infestation and infection with pathogens. Vet. Parasitol. 257, 15–20. https://doi.org/10.1016/j.vetpar.2018.05.002.

Morelli, S., 2021. The indoor cat: do the endoparasites knock at the door?. In: Proceedings of the Conference "XXXI Conference SoIPa & 2021 EDAYA EVENT", Teramo, 16–19 June 2021.

Morelli, S., Diakou, A., Di Cesare, A., Colombo, M., Traversa, D., 2021. Canine and feline parasitology: analogies, differences, and relevance for human health. Clin. Microbiol. Rev. 34, e00266-20. https://doi.org/10.1128/CMR.00266-20.

Ogden, N.H., Cripps, P., Davison, C.C., Owen, G., Parry, J.M., Timms, B.J., Forbes, A.B., 2000. The ixodid tick species attaching to domestic dogs and cats in Great Britain and Ireland. Med. Vet. Entomol. 14, 332–338. https://doi.org/10.1046/j.1365-2915.2000.00244.x.

Pennisi, M.G., Persichetti, M.F., Serrano, L., Altet, L., Reale, S., Gulotta, L., Solano-Gallego, L., 2015. Ticks and associated pathogens collected from cats in Sicily and Calabria (Italy). Parasit. Vectors 8, 512. https://doi.org/10.1186/s13071-015-1128-3.

Persichetti, M.F., Solano-Gallego, L., Serrano, L., Altet, L., Reale, S., Masucci, M., Pennisi, M.G., 2016. Detection of vector-borne pathogens in cats and their ectoparasites in southern Italy. Parasit. Vectors 9, 247. https://doi.org/10.1186/s13071-016-1534-1.

Reinemeyer, C.R., Charles, S.D., Buch, J., Settje, T., Altreuther, G., Cruthers, L., et al., 2005. Evaluation of the efficacy of emodepside plus praziquantel topical solution against ascarid infections (*Toxocara cati* or *Toxascaris leonina*) in cats. Parasitol. Res. 97, S41–S50. https://doi.org/10.1007/s00436-005-1443-2.

Rohdich, N., Zschiesche, E., Wolf, O., Loehlein, W., Pobel, T., Gil, M.J., Roepke, R.K.A., 2018. Field effectiveness and safety of fluralaner plus moxidectin (Bravecto® Plus) against ticks and fleas: a European randomized, blinded, multicenter field study in naturally-infested client-owned cats. Parasit. Vectors 11, 598. https://doi.org/10.1186/s13071-018-3175-z.

Tørnqvist-Johnsen, C., Dickson, S.A., Rolph, K., Palermo, V., Hodgkiss-Geere, H., Gilmore, P., Gunn-Moore, D.A., 2020. First report of Lyme borreliosis leading to cardiac bradydysrhythmia in two cats. JFMS Open Rep. 6, 2055116919898292. https://doi.org/10.1177/2055116919898292.

Traversa, D., Veronesi, F., Danesi, P., Morelli, S., Crisi, P.E., Morganti, G., et al., 2019. Pilot study evaluating the efficacy of a topical formulation containing emodepside and praziquantel in the treatment of natural feline troglostrongylosis. Parasit. Vectors 12, 97. https://doi.org/10.1186/s13071-019-3361-7.

Tulloch, J.S.P., McGinley, L., Sánchez-Vizcaíno, F., Medlock, J.M., Radford, A.D., 2017. The passive surveillance of ticks using companion animal electronic health records. Epidemiol. Infect. 145, 2020–2029. https://doi.org/10.1017/S0950268817000826.

Volk, J.O., Felsted, K.E., Thomas, J.G., Siren, C.W., 2011. Executive summary of the Bayer veterinary care usage study. J. Am. Vet. Med. Assoc. 238, 1275–1282. https://doi.org/10.2460/javma.238.10.1275.

Wright, I., 2018. Current parasitological threats in the UK. Vet. Nurse 9, 63–69. https://doi.org/10.12968/vetn.2018.9.2.63.

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Immediate and long-term efficacy of Felpreva®, a new spot-on formulation containing tigolaner, emodepside and praziquantel, applied as a single application to cats artificially infested with the cat flea *Ctenocephalides felis*

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ABSTRACT

Five studies (two dose determination, two dose confirmation, and one speed of flea kill study) were conducted to assess the immediate (therapeutic) efficacy and long-term persistent (preventive) efficacy of a single spot-on application containing the novel acaricide and insecticide tigolaner in combination with emodepside and praziquantel (Felpreva®, Vetoquinol S.A. Lure, France) applied to cats artificially infested with *Ctenocephalides felis*. Eight cats per group were randomly allocated to 0, 1×, 1.3× and 2× of the minimum dose (14.5 mg/kg body weight) of tigolaner (dose determination studies) or randomly allocated to 0 and 1× of the dosage (dose confirmation studies). Onset of efficacy was assessed in a speed of flea kill study on an existing flea infestation 8, 12 and 24 h after treatment and reassessed after monthly flea reinfestation until 13 weeks post-treatment. Efficacy was calculated according to the Abbott formula using arithmetic means. Efficacy was claimed when (i) control groups were adequately infested (flea retention $\geq$ 50%) at each time-point in the studies; (ii) flea counts in treated groups were significantly lower ($P \leq 0.05$) than flea counts in control groups; and (iii) calculated efficacy was $\geq$ 90% (speed of flea kill study) and $\geq$ 95% (dose determination and dose confirmation studies). Tigolaner at 14.5 mg/kg body weight was 100% effective against fleas on Day 1 (immediate, therapeutic efficacy) in both, dose determination and dose confirmation studies. The long-term persistent efficacy in week 13 ranged between 96.3% and 100%. Fleas were rapidly killed within 12 h after treatment (100% flea reduction, immediate efficacy). New flea infestations were successfully prevented for 8 weeks (98.9–100% flea reduction) within 8 h after reinfestation, and at week 13 (96.3% flea reduction) within 24 h after reinfestation.

1. Introduction

The cat flea *Ctenocephalides felis* is one of the most important ectoparasites found on domestic dogs and cats worldwide (Dryden & Rust, 1994; Rust & Dryden, 1997; Blagburn & Dryden, 2009; Rust, 2017). Being rather host-preferential than host-specific, *C. felis* has been found on numerous hosts including humans and is presumed to infest a wide range of mammalian and avian wildlife (Otranto & Wall, 2008; Rust, 2017; Clark et al., 2018).

Flea bites can cause irritating skin reactions in the infested animal. Initial signs of papules and erythema may become progressively severe and self-traumatic skin lesions may lead to hyperpigmentation, alopecia and pyoderma (Krämer & Mencke, 2001; Noli, 2020). In the allergic animal, intense pruritus and inflammation are typical signs of flea allergy dermatitis (FAD), also called flea bite hypersensitivity (FBH). FAD is a consequence of a hypersensitivity reaction of the animal to certain low molecular allergens in the flea saliva. The immunopathogenesis of the sensitization process is not yet completely understood, but both immediate (type I) and delayed (type IV) hypersensitivity occur in dogs and cats (Dryden & Blakemore, 1989; Lee et al., 1999; Kunkle et al., 2003; Wilkerson et al., 2004). In cats, FAD is one major cause of feline miliary dermatitis (Colombini et al., 2001; Jackson & Foster, 2006; Noli, 2020).

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Chronically infested pets may suffer from anaemia and heavy flea infestations have been known to produce severe iron deficiency anaemia in young animals (Dryden & Gaafar, 1991; Dryden & Rust, 1994; Krämer & Mencke, 2001; Traversa, 2013). Besides the direct pathogenic effects on pets, fleas are also important vectors for a variety of pathogens, some with zoonotic potential such as bacteria *Bartonella henselae* (the causative pathogen of the cat scratch disease) or *Rickettsia felis* (causing cat flea rickettsiosis, flea-borne spotted fever or cat flea typhus). *Ctenocephalides felis* is the intermediate host for the tapeworm *Dipylidium caninum* (Moriello, 2003; Bitam et al., 2010; Halos et al., 2014; Rust, 2017; Rensch & Elston, 2019; ECDC, 2021).

Despite the wide range of commercially available flea products, flea control and management of FAD in pets remains a challenge for both veterinarians and pet owners (Dryden & Blakemore, 1989; Carlotti & Jacobs, 2001; Rust, 2005; Dryden, 2009). Persisting flea infestations are the result of various factors, but often related to the complex life-cycle, the lacking host specificity and the high reproductive capacity of *C. felis*. Modern flea control strategies aim to interrupt the flea life-cycle and prevent flea reproduction. Scientific organisations such as the European Scientific Counsel Companion Animal Parasites (ESCCAP), the Tropical Council for Companion Animal Parasites (TroCCAP) or the Companion Animal Parasite Council (CAPC) therefore recommend continuous flea treatment of pets, depending on the pet's lifestyle, owners' needs, housing situation and the outdoor environment (CAPC, 2017; ESCCAP, 2022; TroCCAP, 2022).

A new spot-on formulation (Felpreva®, Vetoquinol S.A. Lure, France) was recently registered for cats, containing tigolaner, emodepside, and praziquantel at 14.4 mg/kg, 3 mg/kg, and 12 mg/kg body weight, respectively. Tigolaner is a new chemical acaricide and insecticide that acts as antagonist of gamma aminobutyric acid (GABA)- and glutamate-gated chloride channels. Though with the same mode of action, tigolaner is not an isoxazoline, but belongs to the chemical class of bispyrazoles.

This article presents the results of a series of laboratory studies that assessed the immediate (therapeutic) and long-term, persistent (preventive) efficacy of Felpreva® for fleas when applied topically to cats experimentally infested with adult *C. felis*. The objective of these studies was to show that a single treatment with Felpreva® results in a fast onset of flea reduction after treatment and provides a long-term protection against flea reinfestations over a period of 13 weeks.

2. Materials and methods

Two pivotal dose determination studies, two pivotal dose confirmation studies and one speed of flea kill study were conducted. All studies were in compliance with VICH GL 9 Principles of Good Clinical Practice (EMA, 2000) and internal Standard Operating Procedures (SOPs). The studies were designed following the recommendations of the guideline for "Testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats" (EMA, 2016). All studies were part of a development programme for the regulatory approval of Felpreva®.

2.1. Animals and study design

The studies were randomised, blinded, negative controlled studies, using a parallel group design. Study animals were purpose-bred Domestic Shorthair or mixed breed cats (*Felis catus*) of both sexes, between 6 and 122 months of age and with body weights ranging between 2 and 5.8 kg. The cats were housed individually after study inclusion, according to accepted animal welfare guidelines, local animal welfare regulations and Ethics Committee approvals. Standard commercial diets were fed according to the cats' age and nutritional needs. Water was supplied *ad libitum*. Food and water were expected to be free of any contaminants that could interfere with the study.

Cats were acclimatised for at least 7 days and were clinically healthy at study start. None of the cats had been treated with any long-acting topical or systemic acaricide/insecticide for at least 2 months before study inclusion. Individual host suitability was confirmed pre-treatment by infesting the cats with approximately 100 live adult fleas per cat. Fleas were removed and counted approximately 24 h later. Only cats with the highest flea counts were included in the study (flea retention $\geq$ 50%). Cats were blocked on individual pre-treatment flea counts and then randomly allocated to the tigolaner-treated or the negative control groups. Each study group included eight cats (males and females) per group. All personnel involved in flea counting and clinical observation procedures were blinded to treatment allocations.

Body weights (BW) were determined pre-treatment (Day $-1/-2$) for dose calculation purposes and reassessed every month until study end. Physical examinations were performed on the same days. All cats were carefully evaluated for clinical signs on Day 0 before, and 1 h, 4 h and 8 h after treatment application. Clinical exams were continued in regular intervals until study end. Local tolerance observations were conducted shortly before and 1 h, 4 h, 8 h, 24 h and 48 h after spot-on application. General health observations were performed daily throughout the entire study period.

2.2. Treatment administrations

Treatment formulations were fixed combination spot-on formulations containing emodepside, praziquantel and tigolaner. The cats were individually dosed using pre-treatment body weights. With exception of dose determination study #1, dose rates for emodepside and praziquantel were the same in all treatment groups, i.e. 3 mg/kg BW and 12 mg/kg BW, respectively, as it was previously established and registered for Profender® spot-on solution (Vetoquinol S.A., Paris, France). The minimum effective dose for tigolaner was set to 14.5 mg/kg BW (1×-dose). To facilitate an accurate dosing with the fixed combination, dose determination study #2 used treatment formulations in concentrations individually adjusted to the different dosages (Table 1). Intended tigolaner concentrations in dose determination studies were 0.5× (7.25 mg/kg BW), 1× (14.5 mg/kg BW), 1.3× (19.6 mg/kg BW) and 2× (29 mg/kg BW). Dose confirmations studies and the speed of flea kill study used only one tigolaner dosage, i.e. 14.5 mg/kg BW. Dose confirmation study #2 included a non-interference design to assess any possible impact of emodepside and praziquantel on the flea efficacy of tigolaner. Parallel groups of cats were treated with Felpreva®, Profender® or tigolaner mono spot-on.

Application volumes (calculated as BW × application volume per BW) were rounded up to one decimal place. Control cats received technical oil (dose determination studies), Solketal (syn. isopropylidenglycerin), a glycerol derivative (dose confirmation studies), or mineral oil (speed of flea kill study). All products were administered once on Day 0, applied as spot-on formulations directly to the skin at the base of skull of each cat.

2.3. Flea infestations and flea counts

2.3.1. Dose determination and dose confirmation studies

Dose determination and dose confirmation studies were designed to assess the immediate (therapeutic) and long-term persistent (preventative) efficacy. Fleas used in these studies originated from the study facilities' local laboratory reared flea colonies that consisted of European *C. felis* strains, routinely fed on cats and regularly enriched. Each cat was infested with approximately 100 newly emerged, unfed, adult fleas of mixed sex. Fleas were placed before treatment on Day -1 (immediate efficacy) and post-treatment after 4, 8, 9, 10, 11, 12 and 13 weeks (long-term, persistent efficacy). Flea counts were performed approximately 24 ($\pm$ 2) h after each infestation time-point. All body areas of each cat were thoroughly and systematically combed with a fine-tooth flea comb at least twice. When fleas were still present, procedures were repeated for a third time or more until no live fleas were found.

Table 1
Tigolaner dose levels in efficacy studies, with an intended minimum effective dose (1×) of 14.5 mg tigolaner per kg BW in cats artificially infested with the cat flea *Ctenocephalides felis*.

Study	Tigolaner dose	Product	Dose rate per kg BW	Application volume per kg BW
DDS #1	0	Technical oil	na	0.150 ml
	0.5×	Felpreva®	7.25 mg tigolaner +1.5 mg emodepside +6 mg praziquantel	0.075 ml
	1×	Felpreva®	14.5 mg tigolaner +3 mg emodepside +12 mg praziquantel	0.150 ml
	2×	Felpreva®	29 mg tigolaner +6 mg emodepside +24 mg praziquantel	0.300 ml
DDS #2	0	Technical oil	na	0.150 ml
	0.5×	Test formulation 1	7.35 mg tigolaner +3.06 mg emodepside +12.21 mg praziquantel	0.150 ml
	1×	Felpreva®	14.69 mg tigolaner [a] +3.06 mg emodepside +12.21 mg praziquantel	0.150 ml
	1.3×	Test formulation 2	19.6 mg tigolaner +3.02 mg emodepside +12.0 mg praziquantel	0.200 ml
DCS #1	0	Solketal	na	0.148 ml
	1×	Felpreva®	14.5 mg tigolaner +3 mg emodepside +12 mg praziquantel	0.148 ml
DCS #2 [b]	0	Solketal	na	0.148 ml
	0	Profender®	3 mg emodepside +12 mg praziquantel	0.148 ml
	1×	Felpreva®	14.5 mg tigolaner +3 mg emodepside +12 mg praziquantel	0.148 ml
	1×	Tigolaner mono	14.5 mg tigolaner	0.148 ml
Speed of flea kill	0	Mineral oil	na	0.148 ml
	1×	Felpreva®	14.5 mg tigolaner +3 mg emodepside +12 mg praziquantel	0.148 ml

Abbreviations: DDS, dose determination study; DCS, dose confirmation study; BW, body weight; na, not applicable.
[a] By using the final formulation of Felpreva® (20.35 mg emodepside/ml, 81.4 mg praziquantel/ml and 97.9 mg tigolaner/ml) in the 1×-group, 0.15 ml of formulation per kg BW is equivalent to 14.69 mg tigolaner/kg BW. The minimum dose of tigolaner (14.5 mg/kg) was respected.
[b] Dose confirmation study including a non-interference design with parallel groups treated with Felpreva®, Profender® and tigolaner mono spot-on. Efficacy data of Profender® and tigolaner mono spot-on not reported here.

2.3.2. Speed of flea kill study

The onset of efficacy was assessed in a speed of flea kill study. Cats were infested before treatment on Day −2 and again on Days 28, 56 and 91. Flea counts were conducted 8, 12 and 24 h (± 15 min) after treatment (Day 0/1 assessment) and again after 8, 12 and 24 h (± 15 min) following monthly reinfestations with fleas (Day 28/29; Day 56/57; and Day 91/92 assessments) on pairs of tigolaner-treated and negative control groups. Fleas of this study originated from a flea colony provided by Elward II Labs, Soquel, CA, USA.

2.4. Statistical analysis

2.4.1. Dose determination and dose confirmation studies

Live flea counts of each time-point were used to calculate arithmetic means by study day and treatment group. Geometric means (count + 1 data with 1 subsequently subtracted from result) were additionally calculated in dose determination and confirmation studies, but efficacy claims were based on arithmetic means. Adequacy of infestation was demonstrated in the negative control groups when at least six cats were infested with ≥ 50 live fleas (flea retention ≥ 50%) at each time-point. Efficacy (%) was calculated using the Abbott's formula: 100 × (C-T)/C, where C is the arithmetic/geometric mean of live flea counts on cats in the negative control group and T is the arithmetic/geometric mean of live flea counts on cats in the treated groups (Abbott, 1925). Group comparisons were made using a one-way analysis of variance (ANOVA) in SAS 9.3 (SAS Institute Inc., Cary, NC, USA) with a treatment effect, assuming a normal distribution of the data. All hypotheses were tested at a two-sided 0.05 level of significance. Efficacy was claimed when efficacy ≥ 95% was calculated and a statistically significant difference ($P \leq 0.05$) between the treatment group and control group was demonstrated. The experimental unit was the individual cat.

2.4.2. Speed of flea kill study

Live flea counts were analysed with an ANOVA model in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) including treatment as a fixed effect. Efficacy was calculated using least square means and the Abbott's formula as described in *Section* 2.4.1. All hypotheses were tested at a two-sided 0.05 level of significance. Efficacy was claimed when efficacy ≥ 90% was calculated and a statistically significant difference ($P \leq 0.05$) between the treatment group and control group was demonstrated. The experimental unit was the individual cat.

3. Results

3.1. Adequacy of flea infestations

All control groups were adequately infested with fleas at each time-point in all studies, with one exception. On Day 1 in dose determination study #2, only 5 instead of the minimum of 6 control cats were infested with ≥ 50 live fleas. For all other time-points in that study (Day 30, Day 70, Day 86 and Day 94), adequacy of infection was confirmed in all 8, on one occasion in 7 control cats (Day 94). Therefore, the validity of the study was not questioned, and the statistical analyses were proceeded as planned.

3.2. Dose determination studies

In dose determination study #1 (Table 2), tigolaner showed persistent, long-term efficacy in the 0.5× group over the complete study period, with rates of 100% on Day 59, 96.6% on Day 84 and 96.0% at study end on Day 91. In the 1×-group, efficacy was high until 8 weeks after treatment (100% on Day 59). After that, rates declined to 88.2% (Day 84) and 85.2% (Day 91), which was attributed to one outlier cat with high flea counts on those days (63 and 79 fleas, respectively). Geometric means are less affected by outliers and when efficacy was calculated based on geometric means, rates were as high as 97.8% for Day 84 and 97.9% for Day 91. No live fleas were recovered at any time-point in the 2×-group.

In dose determination study #2 (Table 2), persistent efficacy in the 0.5×-group was high for 9 weeks (99.4% on Day 84) but fell below guideline's recommended 95% at study end (93.9% on Day 91) whereas 1.3× of the dose produced consistently high efficacy over the entire study period (100% on Days 59 and 84; 98.9% on Day 91). In the 1×-group, efficacy was high in week 8 (100% on Day 59) and week 13 (98.9% on Day 91), but below the threshold of 95% at the intermediate time-point in week 9 (93.7% on Day 84). The reason was another outlier cat with high flea counts on that day (33 fleas). Efficacy based on geometric means was 99.1%. All other cats in the 1×-group were flea-free throughout the study at all time-points (Day 1, Day 84 and Day 91). Full immediate therapeutic efficacy on Day 1 was seen in all treatment groups (100%).

Flea counts in the tigolaner-treated groups were significantly less ($P < 0.0001$) than in the control groups at all time-points in both studies.

Table 2
Arithmetic (geometric) mean flea counts and calculated percent efficacy against the cat flea *Ctenocephalides felis* for tigolaner-treated groups compared to negative control up to 13 weeks post-treatment in dose determination studies (8 cats per group).

Treatment group tigolaner dose [a]		Immediate efficacy		Long-term persistent efficacy					
		Day 1		Week 8 (Day 59)		Week 9 (Day 84)		Week 13 (Day 91)	
		AM (GM)	Efficacy (%)	AM (GM)	Efficacy (%)	AM (GM)	Efficacy (%)	AM (GM)	Efficacy (%)
DDS #1	0	n.d.	n.d.	68.3 (67.4)	–	73.0 (72.4)	–	77.9 (77.4)	–
	0.5×	n.d.	n.d.	0* (0)	100 (100)	2.5* (0.5)	96.6 (99.4)	3.1* (1.2)	96.0 (98.4)
	1×	n.d.	n.d.	0* (0)	100 (100)	8.6* (1.6)	88.2 (97.8) [b]	11.5* (1.6)	85.2 (97.9) [b]
	2×	n.d.	n.d.	0* (0)	100 (100)	0* (0)	100 (100)	0* (0)	100 (100)
DDS #2	0	64.6 (55.7)	–	n.d.	n.d.	65.8 (64.7)	–	80.5 (79.5)	–
	0.5×	0* (0)	100 (100)	n.d.	n.d.	0.4* (0.2)	99.4 (99.7)	4.9* (0.9)	93.9 (98.9)
	1×	0* (0)	100 (100)	n.d.	n.d.	4.1* (0.6)	93.7 (99.1) [b]	0.9* (0.3)	98.9 (99.6)
	1.3×	0* (0)	100 (100)	n.d.	n.d.	0* (0)	100 (100)	0.9* (0.5)	98.9 (99.3)

* Mean arithmetic flea counts in tigolaner-treated groups were significantly lower than in control groups at all time-points (ANOVA, $P < 0.0001$).
Abbreviations: DDS, dose determination study; AM, arithmetic mean; GM, geometric mean; BW, body weight; n.d., not done.
[a] Tigolaner-emodepside-praziquantel combination applied at intended doses for tigolaner: 0.5× = 7.25 mg/kg BW; 1× = 14.5 mg/kg BW; 1.3× = 18.85 mg/kg BW; 2× = 29 mg/kg BW.
[b] Efficacy influenced by two outlier cats presenting high flea counts: in DDS #1 on Day 84 (63 fleas) and on Day 91 (79 fleas), both caused by the same cat. In DDS #2 on Day 84 (33 fleas) caused by one cat.

3.3. Dose confirmation studies

High, long-term, persistent efficacy of tigolaner at the intended dose of 14.5 mg/kg BW was shown in both dose confirmation studies (Table 3) for a minimum duration of 12 weeks in dose confirmation study #2 (99.5% on Day 86) and up to 13 weeks in dose confirmation study #1 (100% on Day 91). Efficacy was 100% at all time-points in dose confirmation study #1. Full (100%) immediate therapeutic efficacy on Day 1 was seen in both studies.

Flea counts in the tigolaner-treated groups were significantly less ($P < 0.0001$) than in the negative control groups in both studies at all time-points. Non-interference analyses confirmed that emodepside and praziquantel are not effective against fleas (data not shown).

3.4. Speed of flea kill

No live fleas were recovered from any animal (100% efficacy), when flea counts were performed on flea-infested cats 12 and 24 h after treatment. Eight hours after treatment, efficacy was as high as 88.0% which was mostly related to one outlier cat from which 59 fleas were collected on that day.

When efficacy was reassessed after monthly reinfestations with fleas, flea reductions 8 and 12 h after reinfestation were high in week 4 (100% at both time-points) and week 8 (98.9% at 8 h and 99.4% at 12 h) but declined to lower values in week 13 (49.5% at 8 h and 68.8% at 12 h). When assessed after 24 h, efficacy was high throughout the whole study period, i.e. 100% in weeks 4 and 8 and 96.3% in week 13 (Table 4).

Flea counts in the tigolaner-treated groups were significantly less ($P < 0.01$) than in the negative control groups at all time-points.

3.5. Safety observations

A total of 6 adverse events with possible product involvement were recorded. In dose determination study #1, one cat in the 1x-group developed a mild erythema at the application site which did not require any treatment. In dose confirmation study #1, five cats in the Felpreva®-treated group started scratching or tried licking the application site immediately after spot-on application but signs resolved quickly (within 30 min).

4. Discussion

Persisting flea infestations are a common problem for veterinary practitioners, even though the biology and ecology of *C. felis* is well understood. Newly emerged adult female cat fleas (*C. felis*) begin blood-feeding almost immediately after infesting a host and begin egg production 24–48 h later (Krämer & Mencke, 2001). About 70% of the flea eggs dislodge from the pet's fur within eight hours and are spread into the home environment, building large reservoirs for subsequent, almost impossible-to-find immature flea stages (Dryden & Rust, 1994; Halos et al., 2014). Female fleas can stay on the host for several weeks taking multiple blood meals per day and are able to produce up to 40–50 eggs

Table 3
Arithmetic (geometric) mean flea counts and calculated percent efficacy against the cat flea *Ctenocephalides felis* for tigolaner-treated groups compared to negative control groups up to 13 weeks after treatment in dose confirmation studies (8 cats per group).

Week	Day	DCS #1			Day	DCS #2		
		Control	Tigolaner [a] (14.5 mg/kg BW)			Control	Tigolaner [a] (14.5 mg/kg BW)	
		AM (GM)	AM (GM)	Efficacy (%)		AM (GM)	AM (GM)	Efficacy (%)
	Day 1	58.9 (56.2)	0* (0)	100 (100)	Day 1	45.4 (36.8)	0* (0)	100 (100)
Week 3/4	Day 27	65.8 (64.9)	0* (0)	100 (100)	Day 30	74.1 (73.2)	0* (0)	100 (100)
Week 8	Day 56	69.6 (69.4)	0* (0)	100 (100)	Day 58	72.5 (71.8)	0.1* (0.1)	99.8 (99.9)
Week 9	Day 69	70.8 (70.2)	0* (0)	100 (100)	n.d.	–	–	–
Week 10	Day 76	76.6 (75.9)	0* (0)	100 (100)	n.d.	–	–	–
Week 11	Day 83	75.5 (74.9)	0* (0)	100 (100)	n.d.	–	–	–
Week 12	n.d.	–	–	–	Day 86	70.0 (68.8)	0.4* (0.2)	99.5 (99.7)
Week 13	Day 91	81.4 (80.9)	0* (0)	100 (100)	Day 94	65.1 (58.9)	6.9* (3.5)	89.4 (94.1)

Abbreviations: DCS, dose confirmation study; AM, arithmetic mean; GM, geometric mean; BW, body weight; n.d., not done.
* Mean arithmetic flea counts in tigolaner-treated groups were significantly lower than in control groups at all time-points (ANOVA, $P < 0.0001$).
[a] Tigolaner-emodepside-praziquantel combination (Felpreva®) applied at intended doses of 14.5 mg/kg BW tigolaner, 3 mg/kg BW emodepside and 12 mg/kg BW praziquantel.

Table 4
Arithmetic mean flea counts and calculated percent efficacy against the cat flea *Ctenocephalides felis* for tigolaner-treated groups compared to negative control groups at 8, 12 and 24 h post-infestation evaluated 4, 8 and 13 weeks after treatment (speed of flea kill study, 8 cats per group).

Time-point	8 hours				12 hours				24 hours			
	Control	Tigolaner [a]	Efficacy (%)	*P*-value	Control	Tigolaner [a]	Efficacy (%)	*P*-value	Control	Tigolaner [a]	Efficacy (%)	*P*-value
Day 0/1	66.5	8.0	88.0 [b]	<0.0001	58.8	0	100	<0.0001	65.9	0	100	<0.0001
Week 4 (Day 28/29)	69.5	0	100.0	<0.0001	71.8	0	100	<0.0001	68.4	0	100	<0.0001
Week 8 (Day 56/57)	56.1	0.6	98.9	<0.0001	59.0	0.4	99.4	<0.0001	67.8	0	100	<0.0001
Week 13 (Day 91/92)	52.8	26.6	49.5	0.0054	56.9	17.8	68.8	0.0002	61.4	2.3	96.3	<0.0001

[a] Tigolaner-emodepside-praziquantel combination (Felpreva®) applied at intended doses of 14.5 mg/kg BW tigolaner, 3 mg/kg BW emodepside and 12 mg/kg BW praziquantel.
[b] Efficacy influenced by one outlier cat presenting 59 fleas on Day 0 at the 8 h time-point.

per day or 1350 eggs over a 50-day period. Under ideal climate conditions, as in temperate indoor environments, the flea life-cycle may be completed within two to four weeks, releasing the next batch of adult fleas in search for a host (Dryden & Blakemore, 1989; Dryden & Rust, 1994; Cadiergues et al., 2000). Simulated home environment studies have shown that the spot-on flea products of the last two decades are able to effectively control flea infestations on the animal and in the indoor environment. It has been suggested that additional use of insecticide sprays indoors as well as outdoors may no longer be required to control indoor flea populations (Rust, 2017, 2020). Nevertheless, flea-infested pets and FAD are two common diagnoses in veterinary practice. It is estimated that 50% and more of all dermatological cases reported to veterinarians are flea-related (Rust & Dryden, 1997; Beugnet & Franc, 2010; Noli, 2020). Recent investigations on ectoparasiticides purchase transactions and owner surveys regarding compliance to veterinary recommendations on ectoparasite control, have identified the pet owner as one among several key factors. An owner survey in Portugal showed that most dogs (92.2%) but only approximately half (52.7%) of the cats were treated against ectoparasites. Within the two populations only 27.7% of the dogs and 17.2% of the cats received monthly treatments throughout the year (Matos et al., 2015). Other authors found that annual ectoparasiticide purchases of cat owners in the USA covered between 2.8 (for monthly applications) and 4.2 months (for 3-months applications) of flea control. Cat owners typically purchased only one or two treatment doses per year, regardless of the medication's duration of protection (Lavan et al., 2020).

Historically, topical imidacloprid or fipronil treatments provided flea protection in dogs or cats for approximately one month. Most of the lately launched isoxazoline products for cats have a similar duration of action. A single spot-on application of sarolaner and selamectin (Stronghold® Plus for cats, Zoetis) at minimum doses of 1 mg and 6 mg per kg BW demonstrated persistent efficacy (97.7%) over five weeks (Day 35) (Becskei et al., 2017). One treatment with esafoxolaner in combination with eprinomectin and praziquantel (Nexgard® Combo spot-on for cats, Boehringer-Ingelheim Animal Health) at minimum doses of 1.44 mg, 0.48 mg and 10 mg per kg BW, respectively, provided efficacy rates between 95.5% and 99.8% four weeks after treatment (Day 28) and variable efficacy thereafter (Tielemans et al., 2021). A single, oral treatment with lotilaner (Credelio®, Elanco Animal Health) at the minimum dose of 6 mg lotilaner per kg BW has demonstrated full flea efficacy for five weeks and prevented weekly flea reinfestations within eight hours for four weeks (97.8% on Day 35) (Cavalleri et al., 2018). Until now, fluralaner in combination with moxidectin (Bravecto® spot-on for cats, MSD Animal Health) has been the only treatment with an extended flea activity, where a single application of fluralaner and moxidectin at a minimum dose of 40 mg and 2 mg per kg BW respectively showed 99.5–100% efficacy against flea challenges over 13 weeks (Fisara et al., 2019).

With tigolaner, a new acaricide and insecticide with long-term treatment duration for cats (Felpreva®) has been recently introduced into the European market. In the studies presented in this article, a single spot-on application of Felpreva® to cats artificially infested with *C. felis* effectively killed existing fleas and prevented weekly flea reinfestations for up to 13 weeks after treatment. In the dose determination studies, results of Day 84 were influenced to some extent by individual outlier cats (one in each study) which cannot really be explained. But both dose confirmation studies and the speed of flea kill study showed that treatment with Felpreva® provided almost full (99.5%) efficacy consistently over 12 weeks (Day 86) and high efficacy (96.3–100%) over 13 weeks (Day 91). Similar results were also found in a European multicenter field study. When applied to naturally flea-infested, client-owned cats, the overall flea efficacy of Felpreva® was 99.7% on Day 90 (Cvejić et al., 2022). These findings indicate that treatment with Felpreva® has the potential to cover at least three flea generations and during this time treated cats will be continuously protected from reinfestation with newly emerged adult fleas from the environment, indoors as well as outdoors.

Tigolaner demonstrated a very fast onset of activity, this was demonstrated by the pharmacokinetic profile of tigolaner (Mencke et al. under review, this issue), the prevention of tick paralysis caused by *Ixodes holocyclus* (Roeber et al., 2023), and the treatment of ear mite (*Otodectes cynotis*) (Blazejak et al. under review, this issue). A large proportion of fleas is already killed after eight hours (88%) and all fleas (100%) are killed within 12 hours after treatment. At this rate, most fleas will not be able to mate or start egg production. This is faster than seen with comparable products (72.5% after 12 hours for sarolaner; see Becskei et al., 2017) and suggests that treatment with Felpreva® can considerably affect flea reproduction. Prevention of flea reproduction will consequently result in a lower contamination of the environment with immature flea stages. Though fleas must bite and start feeding to be exposed to tigolaner, a fast reduction of the infesting flea population will reduce the number of flea bites and thus exposure to salivary antigens. This will minimise the risk of FAD development and may control FAD symptoms when already present. In the multicenter field study, 16 cats with signs of FAD (pruritus, crusts, papules, erythema, scaling and/or alopecia) were without any signs after treatment with Felpreva® at study end (Cvejić et al., 2022). A fast killing effect can help reduce the risk of flea-transmitted diseases.

It is known from human medicine that long-acting medications have a better patient adherence to medical dosing recommendations and that forgetfulness of patients is one major reason for non-adherence. One hypothesis is that the convenience of a 12-week dosing interval might improve treatment adherence of cat owners compared to monthly treatment applications (Lavan et al., 2020, 2021a, 2021b). While pet owner education must be seen as one important pillar in sustained flea control management in veterinary practice, the combination of an easy-to-use spot-on product for a stress-free management of cats with an extended flea activity for up to 13 weeks can help improve the cat owners' compliance to ectoparasitic treatment recommendations.

5. Conclusions

A single spot-on administration of tigolaner in combination with emodepside and praziquantel (Felpreva®) showed 100% flea reduction one day after treatment (immediate therapeutic efficacy) and prevented

flea reinfestations for up to 13 weeks (long-term persistent efficacy). A rapid onset of activity killed 100% of the fleas within 12 hours after treatment. New flea infestations were successfully prevented within eight hours for eight weeks (98.9%) and within 24 hours for 13 weeks (96.3%).

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Ethical approval

The studies were designed in accordance with the standards of Good Clinical Practice (VICH Guideline 9), and the anti-parasitic guideline for dogs and cats (EMEA/CVMP Guideline 005/2000 Rev. 3). Cats were handled in compliance with the relevant Animal Care and Use/Ethics Committee approvals. Housing of cats complied with the Directive 2010/63/EU of the European Parliament and of the council of 22nd September 2010 on the protection of animals used for scientific purposes (including Annex III "Requirements for establishments and for the care and accommodation of animals"), the German animal protection act and the German welfare regulation for laboratory animals.

CRediT authorship contribution statement

Norbert Mencke: Conceptualization, Funding acquisition, Writing – review & editing. **Katrin Blazejak:** Writing – review & editing. **Gabriele Petry:** Investigation, Methodology, Resources, Supervision. **Hannah Hamburg:** Investigation, Methodology, Resources, Supervision. **Hannah Ringeisen:** Investigation, Methodology, Resources, Supervision. **Tanja N. Knoppe:** Formal analysis, Writing – original draft. **Alta Viljoen:** Investigation, Methodology, Resources, Data curation. **Ashley Smith:** Conceptualization, Methodology, Project administration. **Jennifer Spruill:** Investigation, Methodology, Resources, Supervision.

Declaration of competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hannah Ringeisen, Hannah Hamburg and Gabriele Petry were employees of Bayer Animal Health GmbH, an Elanco Animal Health Company, at the time while the studies reported here were conducted. Katrin Blazejak and Norbert Mencke are employees of Vetoquinol S.A., Paris, France. Tanya N. Knoppe is owner of Vet Advice, Hamburg, Germany. Alta Viljoen is an employee of Clinvet International (Pty) Ltd, Blomfontein, South Africa. Ashley Smith and Jennifer Spruill are employees of Elanco Animal Health, Greenfield, USA.

Data availability

The data supporting the conclusions of this article are included within the article. Raw data generated in the study are confidential.

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References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Becskei, C., Cherni, J.A., Vatta, A.F., King, V.L., Lin, D., Rugg, D., 2017. Efficacy and speed of kill of a new spot-on formulation of selamectin plus sarolaner against flea infestations in cats. Vet. Parasitol. 238 (Suppl. 1), S18–S21. https://doi.org/10.1016/j.vetpar.2017.03.010.

Beugnet, F., Franc, M., 2010. Results of a European multicentric field efficacy study of fipronil-(S) methoprene combination on flea infestation of dogs and cats during 2009 summer. Parasite 17, 337–342. https://doi.org/10.1051/parasite/2010174337.

Bitam, I., Dittmar, K., Parola, P., Whiting, M.F., Raoult, D., 2010. Fleas and flea-borne diseases. Int. J. Infect. Dis. 14, e667–e676. https://doi.org/10.1016/j.ijid.2009.11.011.

Blagburn, B.L., Dryden, M.W., 2009. Biology, treatment, and control of flea and tick infestations. Vet. Clin. North Am. Small Anim. Pract. 39, 1173–1200. https://doi.org/10.1016/j.cvsm.2009.07.001.

Cadiergues, M.C., Hourcq, P., Cantaloube, B., Franc, M., 2000. First blood meal of *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) on cats: Time to initiation and duration of feeding. J. Med. Entomol. 37, 634–636. https://doi.org/10.1603/0022-2585-37.4.634.

CAPC, 2017. CAPC Guidelines. Fleas. Companion Animal Parasite Council. https://capcvet.org/guidelines/fleas/. (Accessed 8 August 2022).

Carlotti, D.N., Jacobs, D.E., 2001. Therapy, control and prevention of flea allergy dermatitis in dogs and cats. Vet. Dermatol. 11, 83–98. https://doi.org/10.1046/j.1365-3164.2000.00204.x.

Cavalleri, D., Murphy, M., Seewald, W., Nanchen, S., 2018. Laboratory evaluation of the efficacy and speed of kill of lotilaner (Credelio™) against *Ctenocephalides felis* on cats. Parasites Vectors 11, 408. https://doi.org/10.1186/s13071-018-2972-8.

Clark, N.J., Seddon, J.M., Šlapeta, J., Wells, K., 2018. Parasite spread at the domestic animal - wildlife interface: Anthropogenic habitat use, phylogeny and body mass drive risk of cat and dog flea (*Ctenocephalides* spp.) infestation in wild mammals. Parasites Vectors 11, 8. https://doi.org/10.1186/s13071-017-2564-z.

Colombini, S., Hodgin, E.C., Foil, F.S., Hosgood, G., Foil, L.D., 2001. Induction of feline flea allergy dermatitis and the incidence and histopathological characteristics of concurrent indolent lip ulcers. Vet. Dermatol. 12, 155–161. https://doi.org/10.1046/j.1365-3164.2001.00243.x.

Cvejić, D., Hellmann, K., Petry, G., Ringeisen, H., Hamburg, H., Farkas, R., 2022. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing emodepside, praziquantel and tigolaner, in treating cats naturally infested with fleas and/or ticks. Curr. Res. Parasitol. Vector-Borne Dis. 2, 100099. https://doi.org/10.1016/j.crpvbd.2022.100099.

Dryden, M.W., 2009. Flea and tick control in the 21st century: Challenges and opportunities. Vet. Dermatol. 20, 435–440. https://10.1111/j.1365-3164.2009.00838.x.

Dryden, M., Gaafar, S., 1991. Blood consumption by the cat flea, *Ctenocephalides felis felis* (Siphonaptera: Pulicidae). J. Med. Entomol. 28, 394–400. https://doi.org/10.1093/jmedent/28.3.394.

Dryden, M.W., Blakemore, J.C., 1989. A review of flea allergy dermatitis in the dog and cat. Companion Anim. Pract. 19, 10–17.

Dryden, M.W., Rust, M.K., 1994. The cat flea: Biology, ecology and control. Vet. Parasitol. 52, 1–19.

ECDC, 2021. Fleas (Siphonaptera) - Factsheet for health professionals. European Centre for Disease Prevention and Control. https://www.ecdc.europa.eu/en/all-topics-z/disease-vectors/facts/fleas-siphonaptera-factsheet-health-professionals. (Accessed 8 August 2022).

EMA, 2000. VICH GL9 Good Clinical Practices. CVMP/VICH/595/98-FINAL. European Medicines Agency. https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl9-good-clinical-practices-step-7_en.pdf. (Accessed 8 August 2022).

EMA, 2016. EMEA/CVMP/EWP/005/2000-Rev.3: Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea Infestation in dogs and cats. European Medicines Agency. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-testing-evaluation-efficacy-antiparasitic-substances-treatment-prevention-tick-flea_en-0.pdf. (Accessed 8 August 2022).

ESCCAP, 2022. Control of ectoparasites in dogs and cats. ESCCAP Guideline 03. 7th ed. European Scientific Counsel Companion Animal Parasites. https://www.esccap.org/uploads/docs/4ce0ad9k_0720_ESCCAP_GL3_English_v17_1p.pdf. (Accessed 8 August 2022).

Fisara, P., Guerino, F., Sun, F., 2019. Efficacy of a spot-on combination of fluralaner plus moxidectin (Bravecto® Plus) in cats following repeated experimental challenge with a field isolate of *Ctenocephalides felis*. Parasites Vectors 12, 259. https://doi.org/10.1186/s13071-019-3512-x.

Halos, L., Beugnet, F., Cardoso, L., Farkas, R., Franc, M., Guillot, J., et al., 2014. Flea control failure? Myths and realities. Trends Parasitol. 5, 228–233. https://doi.org/10.1016/j.pt.2014.02.007.

Jackson, A., Foster, A., 2006. Management of feline miliary dermatitis: A clinical update. Practice 28, 147. https://doi.org/10.1136/inpract.28.3.147.

Krämer, F., Mencke, N., 2001. Flea Biology and Control. Springer-Verlag Berlin and Heidelberg GmbH & Co. K, Berlin, Germany. https://doi.org/10.1007/978-3-642-56609-7.

Kunkle, G.A., McCall, C.A., Stedman, K.E., Pilny, A., Nicklin, C., Logas, D.B., 2003. Pilot study to assess the effects of early flea exposure on the development of flea hypersensitivity in cats. J. Feline Med. Surg. 5, 287–294. https://doi.org/10.1016/s1098-612x(03)00026-3.

Lavan, R., Armstrong, R., Normile, D., Vaala, W., 2020. Adherence to veterinary recommendations for ectoparasiticides purchased by cat owners in the USA. Parasites Vectors 13, 541. https://doi.org/10.1186/s13071-020-04415-5.

Lavan, R.P., Armstrong, R., Newbury, H., Normile, D., Hubinois, C., 2021a. Flea and tick treatment satisfaction, preference, and adherence reported by cat owners in the US, UK, or France who treated their cats with transdermal fluralaner. Open Vet. J. 11, 458–467. https://doi.org/10.5455/ovj.2021.v11.i3.19.

Lavan, R., Normile, D., Armstrong, R., Vaala, W., 2021b. Flea and tick treatment satisfaction, preference, and adherence of US cat owners prescribed topical fluralaner (Bravecto® Topical Solution for Cats). Open Vet. J. 11, 80–88. https://doi.org/10.4314/ovj.v11i1.12.

Lee, S.E., Johnstone, I.P., Lee, R.P., Opdebeeck, J.P., 1999. Putative salivary allergens of the cat flea, *Ctenocephalides felis felis*. Vet. Immunol. Immunopathol. 69, 229–237. https://doi.org/10.1016/s0165-2427(99)00057-4.

Matos, M., Alho, A.M., Owen, S.P., Nunes, T., Madeira de Carvalho, L., 2015. Parasite control practices and public perception of parasitic diseases: A survey of dog and cat owners. Prev. Vet. Med. 122, 174–180. https://doi.org/10.1016/j.prevetmed.2015.09.006.

Moriello, K.A., 2003. Zoonotic skin diseases of dogs and cats. Anim. Health Res. Rev. 4, 157–168. https://doi.org/10.1079/AHRR200355.

Noli, C., 2020. Flea biology, allergy and control. In: Noli, C., Colombo, S. (Eds.), Feline Dermatology. Springer Nature Switzerland AG, Cham, Switzerland, pp. 437–449.

Otranto, D., Wall, R., 2008. New strategies for the control of arthropod vectors of disease in dogs and cats. Med. Vet. Entomol. 22, 291–302. https://doi.org/10.1111/j.1365-2915.2008.00741.x.

Rensch, G.P., Elston, D.M., 2019. What's eating you? Cat flea (*Ctenocephalides felis*) revisited. Cutis 104, 182–183.

Roeber, F., Jackson, C., Mallett, S., Chambers, M., Smith, V., Hume, J., et al., 2023. Efficacy and safety of Felpreva®, a spot- on formulation for cats containing emodepside, praziquantel, and tigolaner against experimental infestation with the paralysis tick *Ixodes holocyclus*. Curr. Res. Parasitol. Vector-Borne Dis. 3, 100123.

Rust, M.K., 2005. Advances in the control of *Ctenocephalides felis* (cat flea) on cats and dogs. Trends Parasitol. 21, 232–236. https://doi.org/10.1016/j.pt.2005.03.010.

Rust, M.K., 2017. The biology and ecology of cat fleas and advancements in their pest management: A review. Insects 8, 118. https://doi.org/10.3390/insects8040118.

Rust, M.K., 2020. Recent advancements in the control of cat fleas. Insects 11, 668. https://doi.org/10.3390/insects11100668.

Rust, M.K., Dryden, M.W., 1997. The biology, ecology, and management of the cat flea. Annu. Rev. Entomol. 42, 451–473. https://doi.org/10.1146/annurev.ento.42.1.451.

Tielemans, E., Buellet, P., Young, D., Viljoen, A., Liebenberg, J., Prullage, J., 2021. Efficacy of a novel topical combination of esafoxolaner, eprinomectin and praziquantel against adult cat flea *Ctenocephalides felis* and flea egg production in cats. Parasite 28, 21. https://doi.org/10.1051/parasite/2021017.

Traversa, D., 2013. Fleas infesting pets in the era of emerging extra-intestinal nematodes. Parasites Vectors 6, 59. https://doi.org/10.1186/1756-3305-6-59.

TroCCAP, 2022. Guidelines for the control of ectoparasites of dogs and cats in the tropics. 1st ed. Tropical Council for Companion Animal Parasites. https://www.troccap.com/2017press/wp-content/uploads/2022/01/TroCCAP-Canine-Feline-Ecto-Guidelines-English-v1.pdf. (Accessed 8 August 2022).

Wilkerson, M.J., Bagladi-Swanson, M., Wheeler, D.W., Floyd-Hawkins, K., Craig, C., Lee, K.W., Dryden, M., 2004. The immunopathogenesis of flea allergy dermatitis in dogs, an experimental study. Vet. Immunol. Immunopathol. 99, 179–192. https://doi.org/10.1016/j.vetimm.2004.02.006.

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Efficacy and safety of Felpreva®, a spot-on formulation for cats containing emodepside, praziquantel and tigolaner against experimental infestation with the Australian paralysis tick *Ixodes holocyclus*

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ABSTRACT

The Australian paralysis tick *Ixodes holocyclus* continues to be a serious threat to companion animals along Australia's east coast. The tick produces a potent neurotoxin which causes a rapidly ascending flaccid paralysis, which if left untreated, can result in the death of the animal. There is currently only a limited number of products registered in Australia for the treatment and control of paralysis ticks in cats. Felpreva® is an effective spot-on combination containing emodepside, praziquantel and tigolaner. To investigate the therapeutic and long-term persistent efficacy of Felpreva® (2.04% w/v emodepside, 8.14% w/v praziquantel and 9.79% w/v tigolaner) against experimental infestation with *I. holocyclus* in cats, two studies were undertaken. Fifty cats were included in the studies on study Day -17. These cats were immunized against paralysis tick holocyclotoxin prior to the study commencing. Immunity to holocyclotoxin was confirmed with a tick carrying capacity (TCC) test conducted prior to treatment. Cats were treated once on Day 0. Group 1 cats were treated with the placebo formulation and Group 2 cats were treated with Felpreva®. Cats were infested on Days -14 (tick carrying capacity test), 0, 28, 56, 70, 84 and 91 (weeks 4, 8, 10, 12 and 13). Ticks were counted on cats 24 h, 48 h and 72 h post-treatment and infestation, except during the tick carrying capacity test when they were counted approximately 72 h post-infestation only. The 24-h and 48-h assessments were conducted without removing the ticks. The ticks were assessed, removed and discarded at the 72-h assessment time-points. Significant differences in total live tick counts at ~24 h, ~48 h and ~72 h post-infestation were observed between the treatment and control group. Differences were significant ($P < 0.05$ to < 0.001) in all instances. Treatment efficacies of 98.1–100% were observed ~72 h post-infestation through to 13 weeks (94 days) post-treatment. These results show that a single application of Felpreva® provides effective treatment and control against induced infestation with paralysis ticks for 13 weeks.

1. Introduction

Infestations with the Australian paralysis tick *Ixodes holocyclus* remain to be a major problem in companion animals in Australia. This tick is clinically the most significant tick species in Australia because it produces a potent neurotoxin (holocyclotoxin) that causes a rapidly ascending flaccid paralysis which can be fatal and each year thousands of cases are reported in dogs and cats (Barker & Walker, 2014; Guernier et al., 2016). Coastal areas with dense bushland and vegetation cover, combined with high humidity, temperate climates throughout most of the year and the availability of suitable bandicoot wildlife hosts create a favorable environment for the tick's survival and development. The distribution of *I. holocyclus* is limited to coastal areas of Australia's east coast and extends from southeastern Victoria, throughout New South Wales to northern Queensland and most cases of tick paralysis are reported in spring and summer (Barker & Walker, 2014).

Approximately three days following attachment, the activity and size of the tick's salivary glands increase which is associated with the release of the holocyclotoxin. The toxin interferes with the presynaptic release of acetylcholine (Chand et al., 2016) in the affected host and a single female

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I. holocyclus tick is enough to cause potential paralysis and death. Clinical signs typically start to develop after ~72 h of feeding and include altered voice, laboured respiration, ascending flaccid paralysis eventually leading to respiratory failure and death (Masina & Broady, 1999). A study that retrospectively investigated the occurrence of tick-induced paralysis in cats that were presented to four emergency clinics in Queensland between 2008 and 2016 reported a total of 2077 cases over this period. Out of these 2077 cases, 273 cats either died or had to be euthanized (Leister et al., 2018). The detection of a single infesting tick can be challenging, and attachment may occur in locations that are difficult to examine such as between the digits, inside the anus, vulva or on the hard palate. These locations are also not protected by topically acting acaricides leaving the hosts susceptible to infestation and the subsequent development of tick-induced paralysis. Therefore, significant advantages can be gained from the use of systemically acting acaricides that also provide protection on areas that are distant from the application site (Baker et al., 2018).

Felpreva® containing the cyclic depsipeptide emodepside in combination with praziquantel, provides effective control of a wide range of helminth parasites including nematodes (roundworms, hookworms and lungworms) and cestodes (Cvejić et al., 2022b; Traversa et al., 2022). Tigolaner offers protection against fleas and ticks for 13 weeks (Cvejić et al., 2022a; Mencke et al., 2023). In the present paper, we report on two efficacy studies that investigated the efficacy of Felpreva® for the control of *I. holocyclus* in cats.

2. Materials and methods

2.1. Study design and animals

Two randomised, negative-controlled, efficacy studies were conducted to determine the therapeutic and long-term persistent efficacy of Felpreva® spot-on (2.04% w/v emodepside, 8.14% w/v praziquantel and 9.79% w/v tigolaner) on cats against experimental infestations of *I. holocyclus*. The studies were carried out between April and November 2019 and were conducted in compliance with the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013), Veterinary International Conference of Harmonization Guidelines (EMA, 2000), the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick Infestation on dogs and cats (Marchiondo et al., 2007) and the Australian Pesticides and Veterinary Medicines Authority (APVMA) Preamble for the WAAVP guidelines for fleas and ticks on dogs and cats (APVMA, 2014). Animals were handled in compliance with Animal Research Authority nos. BAA F 18150 W and BAA F 18186 W issued by the Wongaburra Animal Ethics Committee, and applicable local regulations.

Cats were sourced from the Wongaburra Research Centre cat colony. For each study 26–28 cats were immunized against holocyclotoxin by attaching gradually increasing numbers of ticks to the cats at weekly intervals prior to the study animal phase commencing. The ticks were left on the cats for a maximum period of 3 days. The cats were monitored at least twice daily during the immunization process. Any animal that developed clinical signs of tick paralysis was to have all ticks removed and tick anti-toxin serum given if required. Cats were acclimatized for 17 days. For both studies, domestic cats of mixed breeds (long- and short-haired) and of both sexes and neuter status were used. Cats were between 3 and 10 years of age and had a body weight of 3.5–7.7 kg at the time of study commencement (Day 0). Pre-enrolment veterinary clinical examination was conducted on Day -17 for all cats to confirm good clinical health and suitability for study participation.

Housing of cats complied with the guidelines of the Council of Europe (Cons 123, 2006; Appendix A), as required under Animal Research Establishment accreditation from the New South Wales Department of Primary Industries. Cats were housed in pens with a floor area 1.5 × 3 m, equal areas located inside and outside, that allowed each cat to see neighbouring cats through a transparent door. Cats were housed individually whilst infested with ticks. At times when cats were not infested with ticks, they shared housing with up to two other socially compatible cats within the same treatment group. There was no contact between treatment groups to prevent chemical transfer. Cats were individually housed from Day 0 to Day 5 (Study 1) or Day 6 (Study 2) to allow time for the treatments to dry. The placebo-treated Group 1 cats were tended to first during routine husbandry (e.g. feeding, cleaning) and study procedures (e.g. weighing, tick infestations and tick counts) before the treated Group 2 cats. Cats were fed once daily with a standard feline diet and water was provided *ad libitum*.

2.2. Allocation and treatment

Pre-treatment tick carrying capacity (TCC) test was conducted on Day -14 for allocation purposes and to confirm that cats were sufficiently immunized to holocyclotoxin and free of any residual acaricidal efficacy prior to treatment. On Days -10 (Study 1) or -7 (Study 2) cats were allocated to study groups based on TCC. Cats were ranked in descending order of total live ticks [TOL = Live attached (LA) + Live free (LF)] 72 h post-infestation. Twenty cats were then selected for each study; 10 for Group 1 (placebo-treated) and 10 for Group 2 (Felpreva®-treated). The next two cats were selected as spare Group 1 placebo-treated cats. Cats with the lowest tick carrying capacity were excluded. The 20 selected cats were grouped by coat length (long or short) and 10 replicates of two cats were formed. Each cat was randomly allocated to Group 1 or Group 2. Data was sorted by group and cats were paired according to compatibility (non-random) within treatment group. Pairs of cats were then randomly allocated to pairs of pens within the cattery.

Cats were weighed on Day 0 prior to treatment application. 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 0.148 ml/kg body weight, for Felpreva®. Cats that met the following inclusion criteria were enrolled in the study: (i) clinically healthy, including no abnormal signs at the application site as determined by the attending veterinarian/investigator on Day -17; (ii) not clinically pregnant, not excessively fractious; (iii) between 1 and 10 years of age, > 1 kg and less than 8 kg at time of allocation; (iv) manageable and cooperative with study procedures; (v) not treated with a long-acting topical or systemic acaricide/insecticide for at least 2 months before the start of the study; and (vi) tick carrying capacity greater than that of the 2 lowest animals.

2.3. Source of ticks and cat infestation procedure

Unfed adult female *I. holocyclus* ticks, collected between August 2018 and October 2019 from at least three different localities in the Northern Rivers area of New South Wales and/or south-east Queensland, and Far North Queensland, were used in the studies (Table 1). The ticks were maintained in a dark incubator at optimal conditions of temperature and humidity prior to use.

Each cat was infested by manually attaching a total of 10 adult female ticks to the head, shoulders and mid-back. The majority of ticks were attached to the head and shoulders to simulate the tick's natural predilection for these sites. Ticks were placed at skin level and encouraged to attach by gently tapping them with a finger. When attached, they assumed a head down position with their maxillary palps spread. The hypostome (mouthpart) was not visible. Cats were infested on Days 0 (2 h prior to treatment), 28, 56, 70, 84 and 91. The thermostat in the cattery temperature control system was set to a minimum temperature of 18 °C while cats were infested with ticks. Cats were held indoors following infestation until after the 24-h tick assessments. Placebo-treated cats (Group 1 plus two spares) were infested with 4 ticks each approximately mid-way between experimental infestations (Day 15 and Day 42 for Study 1; Day 14 and Day 43 for Study 2) to maintain immunity to

Table 1
The number of sampling locations and proportions of ticks used in the studies by state.

Study	State	No. of sampling locations	No. of ticks collected per state	Percentage of total
Study 1	New South Wales	24	692	48.00
	South East Queensland	14	525	36.50
	Far North Queensland	6	223	15.50
	Total	44	1440	
Study 2	New South Wales	11	902	61.78
	South East Queensland	5	318	21.78
	Far North Queensland	5	240	16.44
	Total	21	1460	

holocyclotoxin. The two spare placebo-treated cats were infested with 4 ticks each during experimental infestations to maintain immunity to holocyclotoxin on Days 0, 28, 56, 70 and 84. The ticks were removed after 3 days.

2.4. Health observations

The health status of cats was monitored daily during the immunization and acclimatization period and for the entire duration of the study. Cats were subjected to a thorough veterinary examination to confirm suitability for inclusion in the study on Day -17 and were then monitored 3 times daily for general health until study completion. Particular attention was paid to symptoms of tick paralysis including incoordination, hind limb paralysis, paresis, pupillary dilation, reduced appetite, changes in vocalisation, dyspnea and respiratory compromise. Each cat was held for 1 min following treatment administration then observed for 5 min for general behaviour. Clinical observations were made on all cats prior to treatment, at approximately 1 h, 24 h and 48 h following treatment of the last animal. Clinical observations were also performed on Days 7, 28, 56 and 84.

2.5. Parasitological examinations

For the TCC test, ticks were counted and removed on Day -11 (approximately 72 h post-infestation). Tick safety searches were conducted approximately 96 h post-infestation. Ticks were counted on cats 24 h, 48 h and 72 h post-treatment and post subsequent infestations (Table 2). The 24-h and 48-h assessments were carried out without removing the ticks. Ticks were assessed, removed and discarded at the 72-h tick counts. Tick safety searches were conducted approximately 96 h post-infestation. Tick safety searches were a precaution to reduce the risk of potential tick paralysis from stray or missed ticks. Any ticks identified during the tick safety searches were removed and discarded. The tick safety searches were not time-dependant and ticks found at this timepoint were not included in the assessment of efficacy. Ticks were counted on cats of one study group at a time, to reduce the potential for chemical transfer between groups. The attachment locations used during the experimental infestations were inspected first, followed by a full body search. Ticks were located by digital palpation. In areas of sparse or short hair (e.g. inner ears, lips and groin) the ticks were located by visual inspection.

Ticks were classified according to viability (live or dead) and attachment status. Attached ticks (A) had their hypostome embedded into the skin of the cat and were not easily dislodged from the cat. Free ticks (F) were unattached ticks. They may have been live and moving through the coat, or dead and sitting in the hair. The ticks found on the cats were assessed using the parameters outlined in Table 3.

Classification was a subjective process undertaken by a suitably experienced tick assessor. Live (L) ticks demonstrated active leg movement, normal engorgement and no crenation. Inflammation and exudate (oozing serum) may have been observed around the attachment site. Tick

Table 2
Detailed study schedule for Study 1. Critical activities that were performed to determine the acaricidal efficacy of Felpreva® are highlighted in bold.

Study day	Activity
Pre-study	Immunization of cats
-17	Veterinary examinations all study cats
	Commence three times daily monitoring
-16 to -15	Monitor cats
-14	TCC infest cats
-13 to -12	Monitor cats
-11	TCC count and remove ticks
-10	96-h tick safety search
	Allocate
-9 to -8	Monitor cats
-7	Re-pen cats
-6 to -1	Monitor cats
0	**Weigh each cat prior to infestation**
	Infest cats with 10 ticks each (including spare placebo treated cats with 4 ticks each)
	Pre-treatment clinical observations
	Treat. Hold cats for 1 min post-treatment administration
	Observe each cat for 5 min post-treatment
	1-h post-treatment clinical observations
1	24-h post-treatment clinical observations
	24-h post-treatment tick assessment
2	48-h post-treatment clinical observations
	48-h post-treatment tick assessment
3	**72-h post-treatment tick assessment and remove**
4	96-h tick safety search
5 to 6	Monitor cats
7	Clinical observations
	Deworm placebo treated cats
8 to 14	Monitor cats
15	Infest placebo treated cats with 4 ticks each to maintain immunity
16 to 17	Monitor cats
18	Remove immunising ticks
19	96-h tick safety search
20 to 27	Monitor cats
28	Clinical observations
	Infest cats with 10 ticks each (including spare placebo-treated cats with 4 ticks each)
29	**24-h post-infestation tick assessment**
30	**48-h post-infestation tick assessment**
31	**72-h post-infestation tick assessment and remove**
32	96-h tick safety search
33 to 41	Monitor cats
42	Infest placebo treated cats with 4 ticks each to maintain immunity
43 to 44	Monitor cats
45	Remove immunising ticks
46	96-h tick safety search
47 to 55	Monitor cats
56	Clinical observations
	Infest cats with 10 ticks each (including spare placebo-treated cats with 4 ticks each)
57	**24-h post-infestation tick assessment**
58	**48-h post-infestation tick assessment**
59	**72-h post-infestation tick assessment and remove**
60	96-h tick safety search
61 to 69	Monitor cats
70	**Infest cats with 10 ticks each (including spare placebo-treated cats with 4 ticks each)**
71	**24-h post-infestation tick assessment**
72	**48-h post-infestation tick assessment**
73	**72-h post-infestation tick assessment and remove**
74	96-h tick safety search
75 to 83	Monitor cats
84	Clinical observations
	Infest cats with 10 ticks each (including spare placebo treated cats with 4 ticks each)
85	**24-h post-infestation tick assessment**
86	**48-h post-infestation tick assessment**
87	**72-h post-infestation tick assessment and remove**
88	96-h tick safety search
89 to 90	Monitor cats
91	**Infest cats with 10 ticks each**

(*continued on next page*)

Table 2 (*continued*)

Study day	Activity
92	**24-h post-infestation tick assessment**
93	**48-h post-infestation tick assessment**
94	**72-h post-infestation tick assessment and remove**
95	96-h tick safety search

Table 3
Tick classification according to viability and attachment status. Adapted from Marchiondo et al. (2013).

Survival status	Attachment status	Abbreviation	Interpretation
Live	Free	LF	Acaricidal effect not demonstrated
Live	Attached	LA	Acaricidal effect not demonstrated
Dead	Free	DF	Acaricidal effect demonstrated
Dead	Attached	DA	Acaricidal effect demonstrated

Abbreviations: L, live; F, free; D, dead; A, attached.

faeces may also have been present. Dead (D) ticks showed no leg movement, did not react when stimulated, and may have appeared crenated or desiccated. Moribund (M) ticks were those that were classified as being dead on the cat, but then displayed feeble leg movement after removal from the cat. Moribund ticks were recorded as LA but were noted to be moribund. Moribund ticks are considered incapable of causing tick paralysis but were included in the live tick count as per APVMA requirements. The total live count (TOL) consisted of all live ticks found on a cat.

2.6. Efficacy assessments and statistical methods

The total number of live ticks (TOL) was used in the calculation of efficacy. Efficacy was calculated based on arithmetic and geometric mean TOL. Treatment effects for 'Efficacy' were calculated in all instances using TOL tick count counts 24 h, 48 h and 72 h post-infestation and the formula: Treatment effect (%) = (Mean Placebo count – Mean Felpreva count)/Mean Placebo count.

All health and clinical observations/examinations were evaluated clinically but were not statistically analysed.

3. Results

3.1. Inclusion criteria, health observations and safety assessments

Out of 24 (Study 1) and 26 (Study 2) cats screened during pre-enrolment veterinary examination and TCC, 20 cats were enrolled in the study based on highest tick counts. For each study, two cats were selected as spares and cats with the lowest tick counts were excluded from the study. There were four adverse events recorded during each of the two studies which were mild in nature and unrelated to the treatment with Felpreva®. Recorded adverse events included sneezing and nasal discharge, swelling on the forehead or of the eye, areas of alopecia, moist dermatitis and skin reddening. These adverse events were associated with tick attachments and usually resolved without any intervention.

3.2. Statistical analysis

A preliminary data exploration was conducted prior to statistical analyses; summary statistics of tick counts and bodyweights prior to treatment. Pre-treatment TOL tick counts appeared to be approximately normally distributed within the overall group of selected cats, with similar median and mean values. When standard deviations were expressed as a percentage of the group mean (coefficient of variation) they were 25–32% (Study 1) or 31–35% (Study 2), indicating relatively moderate variability in the data. Homogeneity of variances for untransformed and log-transformed TOL tick counts post-infestation were tested using Levene's test (calculated using Statistix 10.0, Analytical Software 2013), to determine the suitability of parametric tests (one-way analysis of variance, ANOVA) for comparison of group means. Log-transformation of the data appeared to offer an advantage relative to untransformed (raw) data according to Levene's test results and a slightly improved Shapiro-Wilks normality test *P*-value, hence TOL counts were log-transformed for statistical comparisons. Parametric ANOVA was used to compare TOL counts at allocation and bodyweights prior to treatment, using fixed-effects linear models and the statistical package Spotfire S + Version 8.2, Tibco Software Inc. 2010:

$$TOL.Allocation \sim Treatment + Coat + Sex + Age$$

$$Weight.Day0 \sim Treatment + Replicate + Coat + Sex + Age$$

Post-treatment TOL tick counts were compared using the same package and the fixed-effects linear model:

$$(Count) \sim Treatment + Replicate + Weight.Day0 + Coat + Sex + Age$$

Group mean tick counts were compared at a family-wise significance level of $P < 0.05$ using Tukey's multiple comparison test, with results of pairwise group comparisons presented as confidence intervals. Residuals output was generally acceptable and terms in the model tended to be non-significant with the exception of *Treatment.Group*. Means, medians, standard deviations and coefficients of variation were calculated to assess the normality (or otherwise) of study data.

3.3. Acaricidal efficacy

For both studies, TOL tick counts prior to treatment were similar for both groups, with no significant differences observed at $P < 0.05$. Treatment groups could therefore be considered equivalent prior to treatment. Post-treatment, mean tick counts in placebo-treated cats ranged from 4.9 to 7.7 (mean = 6.3; standard deviation, SD = 0.76) in Study 1 and from 5.2 to 7.0 (mean = 6.1, SD = 0.55) in Study 2 at each sampling time-point and showed that tick infestation was adequate on placebo-treated cats and that trial results can be used to determine treatment efficacy (Table 4). The mean tick counts in Felpreva®-treated cats ranged from 0 to 4.6 (mean = 0.6, SD = 1.06) in Study 1 and from 0 to 3.1 (mean = 0.4, SD = 0.70) in Study 2, and were significantly lower compared to mean tick counts on placebo-treated cats and for each time-point post-treatment or post-infestation. For Study 1, highly significant differences in TOL tick counts were observed between the two groups at ~24 h ($P < 0.05$ to < 0.001), ~48 h ($P < 0.001$) and ~72 h ($P < 0.001$) at all time-points during the study. For Study 2, highly significant differences in TOL tick counts were observed between the two groups at ~24 h ($P < 0.001$) apart from the first occasion (Day 1, $P = 0.038$, however, confidence intervals spanned zero), ~48 h ($P < 0.001$) and ~72 h ($P < 0.001$) at all time-points during the study.

Across the two studies, the acaricidal efficacy of Felpreva® was between 37.0% (Study 1, Day 1) and 98.6% (Study 2, Day 29) at 24 h and between 89.1% (Study 1, Day 2) and 100% (Study 1, Day 30) at 48 h. In Study 1, the acaricidal efficacy of Felpreva® against *I. holocyclus* at the 72-h assessment reached 100% on Days 3, 31, 59, 73, was 98.2% on Day 87, and again reached 100% on Day 94. In Study 2, the acaricidal efficacy of Felpreva® at the 72-h assessments reached 100% on Days 3, 31, 59, 87, and 98.1% on Days 73 and 94 (Table 4). The efficacy of Felpreva® was > 95% (range 98.1–100%) at all 72-h time-points during both studies. This is within the critical period before toxin production takes place and clinical signs of tick paralysis start to develop.

Table 4
The treatment efficacies based on the arithmetic mean Ixodes holocyclus tick counts in Felpreva® and placebo-treated cats at 24 h, 48 h and 72 h following experimental infestation.

Time following treatment (days)	Time after treatment or reinfestation (hours)	Study 1			Study 2		
		Placebo	Felpreva®	Efficacy (%)	Placebo	Felpreva®	Efficacy (%)
1	24	7.3	4.6	37.0	6.1	3.1	49.2
2	48	6.4	0.7	89.1	5.7	0.1	98.2
3	72	5.7	0	100	5.4	0	100
29	24	6.3	0.1	98.4	6.9	0.1	98.6
30	48	5.9	0	100	6.7	0.1	98.5
31	72	5.3	0	100	6.4	0	100
57	24	7.4	0.8	89.2	7.0	0.2	97.1
58	48	6.4	0	100	6.3	0.2	96.8
59	72	6.2	0	100	5.8	0	100
71	24	7.0	0.7	90.0	6.6	0.8	87.9
72	48	6.2	0	100	5.7	0.5	91.2
73	72	5.6	0	100	5.2	0.1	98.1
85	24	7.7	1.2	84.4	6.8	0.3	95.6
86	48	6.8	0.2	97.1	5.9	0	100
87	72	5.7	0.1	98.2	5.5	0	100
92	24	7.1	1.2	83.1	6.4	0.6	90.6
93	48	5.6	0.4	92.9	5.7	0.2	96.5
94	72	4.9	0	100	5.4	0.1	98.1

4. Discussion

The effective control of ectoparasites (ticks and fleas) in cats is of major importance in veterinary practice as well as for pet owners. Both, ticks as well as fleas are known vectors for a variety of pathogens causing vector-borne disease in companion animals. In addition to infections with bacterial, viral or protozoon pathogens, infestations with the Australian paralysis tick can cause life-threatening paralysis and if untreated, result in the death of the animal. Treatment of affected cats often requires intensive emergency critical care and hospitalisation and can be very costly to the owner. *Ixodes holocylus* is not the only tick capable of producing a potent toxin and cases of tick paralysis have also been reported from other continents, as for example in Europe, where mortalities of dogs have been reported as a result of tick paralysis induced by *Rhipicephalus sanguineus* (Otranto et al., 2012). Therefore, the best approach to the control of this parasite is the treatment with effective acaricides that kill the ticks before they release their toxins *via* saliva and thus before clinical signs of paralysis occur. Currently, there is only a limited number of registered products in Australia that offer effective control of *I. holocyclus* in cats. Topically distributed acaricides available for cats are available as sprays, shampoos or collars. Sprays and shampoos, depending on the active substances and concentrations only provide control for a limited period of time (3 days–3 weeks) and require frequent reapplication which represents a challenge to owner compliance and increases the likelihood of cats being exposed to the parasite if treatment intervals are not stringently followed. There is currently only one collar containing flumethrin and imidacloprid registered in Australia for cats which repels and controls paralysis ticks for up to 8 months. However, collars can also be easily lost and topically acting and distributed acaricides may have limited effect on ticks that attach in obscure locations. Therefore, systemically acting acaricides that are topically applied provide an effective and easy-to-use approach for the control of paralysis ticks in cats. Also, products that offer a longer duration of protection have been suggested to increase owner compliance as less frequent reapplication of the treatment is required (Lavan et al., 2017). At present, there are only four such registered products available for cats in Australia which provide protection for 5 weeks to 3 months (reviewed by Roeber & Webster, 2021). Two of these products also contain macrocyclic lactones for added treatment of nematode infections but none of these products contains an active for the treatment of tapeworms. Felpreva® is the first combination product for cats that can be topically applied but is systemically distributed and offers long-lasting (up to three months) protection against paralysis ticks and also contains actives for the control of nematodes and cestodes (emodepside and praziquantel). The efficacy of Felpreva® against experimental *I. holocyclus* infestations was > 95% at all 72-h time-points during both studies which confirms that Felpreva® is effective in the treatment of *I. holocyclus* infestations and kills ticks before clinical signs of tick paralysis can develop. However, regardless of high efficacy achieved, no product will be fully effective on all occasions and an effective *I. holocyclus* protective strategy will also require owners and veterinarians to remain vigilant and ensure that treatment intervals are followed, and regular tick searches are being conducted in animals living in high-risk areas.

There were four adverse events reported during both studies which were mild in nature and related to tick attachment reactions. There were no adverse reactions observed to the treatment with Felpreva® itself, confirming that the treatments were well tolerated in all animals.

5. Conclusions

In conclusion, the combination of a highly effective systemically acting bispyrazole acaricide together with the endoparasiticides emodepside and praziquantel, Felpreva® represents a convenient all-in-one solution for the treatment and control of ecto- and endoparasites in cats. Efficacy of > 95% was demonstrated for three months following treatment which provides an extended time of protection and reduces the number of re-applications, thus increasing owner compliance.

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Ethical approval

The studies were carried out between April and November 2019 and were conducted in compliance with the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). Housing of cats complied with the guidelines of the Council of Europe (Cons 123, 2006; Appendix A), as required under Animal Research Establishment accreditation from the New South Wales Department of Primary Industries. Animals were handled in compliance with Animal Research Authority nos. BAA F 18150 W and BAA F 18186 W issued by the Wongaburra Animal Ethics Committee, and applicable local regulations.

CRediT authorship contribution statement

Florian Roeber: Investigation, Methodology, Resources, Supervision, Writing – original draft. **Chrissie Jackson:** Investigation, Methodology, Resources, Supervision. **Michael Chambers:** Data curation. **Veronica Smith:** Conceptualization, Methodology, Project administration. **Jane Hume:** Writing – review & editing. **Katrin Blazejak:** Writing – review & editing. **Norbert Mencke:** Conceptualization, Funding acquisition, Writing – review & editing. 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: Florian Roeber, Chrissie Jackson and Michael Chambers are employees of Invetus Pty Ltd. Veronica Smith is an employee of Animal Ethics Pty Ltd. Jane Hume is an employee of Vetoquinol Australia. Katrin Blazejak and Norbert Mencke are employees of Vetoquinol S.A.

Data availability

The data supporting the conclusions of this article are included within the article. Raw data generated during the study are confidential.

References

APVMA, 2014. Preamble for the WAAVP guideline for fleas and ticks on dogs and cats. Australian Pesticides and Veterinary Medicines Authority, Canberra. https://apvma.gov.au/node/1040 (Accessed 5 January 2023).

Baker, K., Ellenberger, C., Murphy, M., Cavalleri, D., Seewald, W., Drake, J., Nanchen, S., Hacket, K., 2018. Laboratory evaluations of the 3-month efficacy of oral lotilaner (Credelio™) against experimental infestations of dogs with the Australian paralysis tick, *Ixodes holocyclus*. Parasites Vectors 11, 487. https://doi.org/10.1186/s13071-018-3061-8.

Barker, S.C., Walker, A.R., 2014. Ticks of Australia. The species that infest domestic animals and humans. Zootaxa 3816, 1–144. https://doi.org/10.11646/zootaxa.3816.1.1.

Chand, K.K., Lee, K.M., Lavidis, N.A., Rodriguez-Valle, M., Ijaz, H., Koehbach, J., et al., 2016. Tick holocyclotoxins trigger host paralysis by presynaptic inhibition. Sci. Rep. 6, 29446. https://doi.org/10.1038/srep29446.

Cons 123, 2006. Appendix A of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123). Council of Europe, Strasbourg. https://www.coe.int/t/e/legal_affairs/legal_co-operation/biological_safety_and_use_of_animals/laboratory_animals/2006/Cons123(2006)3AppendixA_en.pdf.

Cvejić, D., Hellmann, K., Petry, G., Ringeisen, H., Hamburg, H., Farkas, R., et al., 2022a. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing emodepside, praziquantel and tigolaner, in treating cats naturally infested with fleas and/or ticks. Curr. Res. Parasitol. Vector-Borne Dis. 2, 100099. https://doi.org/10.1016/j.crpvbd.2022.100099.

Cvejić, D., Mencke, N., Petry, G., Ringeisen, H., Hamburg, H., Hellmann, K., et al., 2022b. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing tigolaner, emodepside and praziquantel, in treating cats with mixed infection with intestinal nematodes, cestodes and/or lungworms. Curr. Res. Parasitol. Vector-Borne Dis. 2, 100098. https://doi.org/10.1016/j.crpvbd.2022.100098.

EMA, 2000. VICH GL9 Good Clinical Practices, July 2000. In: European Medicines Agency, Committee for Medicinal Products for Veterinary Use. https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl9-good-clinical-practices-step-7_en.pdf.

Guernier, V., Milinovich, G.J., Bezerra Santos, M.A., Haworth, M., Coleman, G., Soares Magalhaes, R.J., 2016. Use of big data in the surveillance of veterinary diseases: Early detection of tick paralysis in companion animals. Parasites Vectors 9, 303. https://doi.org/10.1186/s13071-016-1590-6.

Lavan, R.P., Tunceli, K., Zhang, D., Normile, D., Armstrong, R., 2017. Assessment of dog owner adherence to veterinarians' flea and tick prevention recommendations in the United States using a cross-sectional survey. Parasites Vectors 10, 284. https://doi.org/10.1186/s13071-017-2217-2.

Leister, E., Morton, J., Atwell, R., Webster, R., 2018. Clinical presentations, treatments and risk factors for mortality in cats with tick paralysis caused by *Ixodes holocyclus*: 2077 cases (2008–2016). J. Feline Med. Surg. 20, 465–478. https://doi.org/10.1177/1098612X17733628.

Marchiondo, A.A., Holdsworth, P.A., Fourie, L.J., Rugg, D., Hellmann, K., Snyder, D.E., Dryden, M.W., 2013. World Association for the advancement of veterinary parasitology (W.A.A.V.P.) second edition: guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats. Vet. Parasitol. 194, 84–97. https://doi.org/10.1016/j.vetpar.2013.02.003.

Marchiondo, A.A., Holdsworth, P.A., Green, P., Blagburn, B.L., Jacobs, D.E., 2007. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestation on dogs and cats. Vet. Parasitol. 145, 332–344. https://doi.org/10.1016/j.vetpar.2006.10.028.

Masina, S., Broady, K.W., 1999. Tick paralysis: development of a vaccine. Int. J. Parasitol. 29, 535–541. https://doi.org/10.1016/S0020-7519(99)00006-5.

Mencke, N., Blazejak, K., Petry, G., Hamburg, H., Ringeisen, H., Knoppe, T.N., et al., 2023. Immediate and long-term efficacy of Felpreva®, a new spot-on formulation containing tigolaner, emodepside and praziquantel applied as a single application to cats artificially infested with the cat flea *Ctenocephalides felis*. Curr. Res. Parasitol. Vector-Borne Dis. 3, 100122. https://doi.org/10.1016/j.crpvbd.2023.100122.

NHMRC, 2013. Australian code for the care and use of animals for scientific purposes, 8th ed. National Health and Medical Research Council, Canberra. https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes. (Accessed 5 January 2023).

Otranto, D., Dantas-Torres, F., Tarallo, V.D., Ramos, R.A.N., Stanneck, D., Baneth, G., Caprariis, D., 2012. Apparent tick paralysis by *Rhipicephalus sanguineus* (Acari: Ixodidae) in dogs. Vet. Parasitol. 188, 325–329. https://doi.org/10.1016/j.vetpar.2012.04.005.

Roeber, F., Webster, M., 2021. Protecting dogs and cats against the Australian paralysis tick, *Ixodes holocyclus* (Acari: Ixodidae): A review of the Australian acaricide registration process. Curr. Res. Parasitol. Vector-Borne Dis. 1, 100054. https://doi.org/10.1016/j.crpvbd.2021.100054.

Traversa, D., Morelli, S., Di Cesare, A., Strube, C., Raue, K., Bisterfeld, K., et al., 2022. Efficacy of two topical combinations containing emodepside plus praziquantel, and emodepside plus praziquantel plus tigolaner, for the treatment of troglostrongylosis in experimentally infected cats. Curr. Res. Parasitol. Vector-Borne Dis. 2, 100097. https://doi.org/10.1016/j.crpvbd.2022.100097.

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Plasma pharmacokinetics of tigolaner, emodepside, and praziquantel following topical administration of a combination product (Felpreva®) and of intravenous administration of the individual active ingredients in cats

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ABSTRACT

Felpreva® for cats contains the new acaricidal/insecticidal active ingredient tigolaner in a fixed combination with the nematocidal and cestocidal compounds emodepside and praziquantel, respectively. The plasma pharmacokinetics of tigolaner, emodepside, and praziquantel were evaluated in clinically healthy cats following topical (spot-on) treatment as fixed combination Felpreva®. For the determination of bioavailability intravenous administration of single active ingredients was also performed. After a single topical administration of Felpreva® using the target dose volume of 0.148 ml/kg to cats, tigolaner reached mean peak concentrations of 1352 μg/l with a T_{max} of 12 days and a mean half-life of 24 days. Simulation of repetitive topical administration every 91 days indicates only a low risk of accumulation after reaching steady state within two to three administrations. The volume of distribution calculated after intravenous dosing was 4 l/kg and plasma clearance was low with 0.005 l/h/kg. Overall plasma exposure was 1566 mg*h/l after topical administration, providing an absolute bioavailability of 57%. Tigolaner was mainly cleared *via* the faeces (54% within 28 days), renal clearance was neglectable (< 0.5% within 28 days). Emodepside and praziquantel showed mean peak concentrations of 44 μg/l and 48 μg/l (reached after 1.5 days and 5 h, respectively). Overall plasma exposures were 20.6 and 3.69 mg*h/l, respectively. The elimination half-life was 14.5 days for emodepside and 10 days for praziquantel after topical administration. After topical administration of Felpreva® using 2.5× and 5× dose multiples an almost proportional increase of plasma exposure was observed for all three active ingredients. With the addition of tigolaner, Felpreva® combines the established pharmacokinetic (PK) characteristics of emodepside and praziquantel contained in Profender® spot-on for cats with the favourable PK of tigolaner suitable for a 3-months protection against fleas and ticks.

1. Introduction

Felpreva® containing tigolaner as a new active ingredient (AI), in combination with the well-established nematocide emodepside and the cestocide praziquantel is a new commercially available treatment and protection against infestations with fleas (*Ctenocephalides felis*), ticks (*Ixodes ricinus*, *Ixodes holocyclus*) and mites (*Notoedres cati*, *Otodectes cynotis*), as well as infections with lungworms (*Aelurostrongylus abstrusus*, *Troglostrongylus brevior*), gastrointestinal nematodes (*Toxocara cati*, *Toxascaris leonina*, *Ancylostoma tubaeforme*) and cestodes (*Dipylidium caninum*, *Taenia taeniaeformis*), providing safe, rapid and long-acting efficacy in cats following a single spot-on administration. Felpreva is indicated when ectoparasites, cestodes and nematodes are to be treated at the same time. The volume to apply dermally is 0.37 ml for a small cat (1.0–2.5 kg), 0.74 ml for medium-sized cats (2.6–5.0 kg) and 1.18 ml for large cats (5.1–8.0 kg). Felpreva is licensed in Europe since November 2021.

Tigolaner, from the bis-pyrazole class of compounds has potent antiparasitic properties acting against γ-aminobutyric acid- (GABA-) and glutamate-gated chloride channels with significant selectivity for insect

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neurons over mammalian neurons (EMA, 2022). Tigolaner has a high potency against insects and acarids by exposure *via* their feeding, i.e. fleas and ticks that initiate feeding will be exposed to the AI (Cvejić et al., 2022a, b; Mencke et al., 2023). A single tigolaner dose administered topically at the minimum recommended dose of 14.5 mg/kg body weight (BW) on cats provides 13 weeks of flea and tick control (Cvejić et al., 2022a, b; Mencke et al., 2023). A fast onset of flea efficacy, the so called speed of flea kill is of clinical relevance to reduce exposure to flea saliva (flea allergic dermatitis) and transmission of pathogens (vector-borne diseases). Studies clearly demonstrated the fast onset of efficacy within 12 h with respect to fleas that are already on the cat prior to treatment. For new flea infestation the onset was within 8 h for two months and within 24 h afterwards (EMA, 2022; Mencke et al., 2023). The fast onset together with the long duration of activity after a single topical administration offers a convenient alternative to monthly flea and tick control treatments and is expected to increase pet owner compliance (Lavan et al., 2020, 2021). Increased compliance assists in limiting protection gaps that can occur with missed re-administration of monthly treatments. Felpreva® is effective in treatment of an existing infestation with ear (*Otodectes cynotis*) and head mange (*Notoedres cati*) mites in a single spot-on application (EMA, 2022) and prevent tick paralysis caused by *Ixodes holocyclus* (Roeber et al., 2023).

Emodepside and praziquantel are already combined in Profender® spot on for cats, which is a well-established helminth protection and treatment with a broad spectrum of activity against both nematodes and cestodes (Altreuther et al., 2005; Taweethavonsawat et al., 2013). With the addition of tigolaner in Felpreva® the therapeutic range is extended to a reliable flea and tick control (Cvejić et al., 2022a).

The present study focusses on the pharmacokinetic (PK) profile of tigolaner, emodepside and praziquantel in cats following a single topical administration of a fixed combination (Felpreva®) with regard to the absorption, distribution, metabolism and elimination. Additionally, it provides insight into possible dose proportionality of the AIs. In addition, a simulation of repeated tigolaner administration (every 91 days) was performed to reveal information about possible cumulation potential. This is of particular interest, as 8-week intervals reveal a possible cumulative behaviour of tigolaner, leading to the Summary of Product Characteristics (SPC) advice that the product should not be administered at intervals shorter than 8 weeks.

2. Materials and methods

2.1. Overview of studies

Altogether three studies were conducted. Cats were treated with Felpreva® (containing emodepside praziquantel and tigolaner) at the recommended dose (14.5 mg/kg tigolaner, 3 mg/kg emodepside and 12 mg/kg praziquantel) or at 2.5× and 5× the recommended dose. For intravenous (i.v.) injection (Study 1) tigolaner, emodepside and praziquantel were formulated in tetraglycol as 8.9%, 1.85% and 7.4% solutions, respectively.

In each study all animal husbandry and study conduct were compliant with local regulations including the Directive 2010/63/EU of the European Parliament and of the Council of 22nd September 2010 on the protection of animals used for scientific purposes. Studies 1 and 2 were performed in Germany and the study design and the experimental procedures had been approved by the responsible authorities (LANUV - Regional authority for nature, environment and consumer protection in North Rhine Westphalia). Study 3 was performed in the Netherlands and approved by the Central Authority for Scientific Procedures on Animals (CCD) as required by the Dutch Act on Animal Experimentation.

2.2. In vivo phase

Table 1 provides animal details for each of the 3 studies and Table 2 presents the study designs. All cats were healthy and acclimatized for a minimum of 7 days. In studies 1 and 2, cats were individually housed for 8 h or 10–12 days following i.v. or topical administration, respectively, to avoid potential cross-contamination between animals. After this period, cats were group-housed by treatment group and sex. In Study 3, cats were socially housed in groups of 3 (same sex/same dose group) in one or more connected similar cages. The exception was when cats were separated for designated study procedures/activities associated with dosing or urine and faeces collection.

Cats in studies 1 and 2 had daily individual social contact with their caretaker while in Study 3 cats were offered enrichment with toy balls. Room environment was monitored continuously in the studies, with a maximum temperature of 24 °C. Relative humidity ranged from 30% to 70%. Cages were cleaned daily with routine hygiene measures in place. Cats were fed once daily with a standard diet suitable for adult cats (Studies 1 and 2: commercial dry feed, Josera Kleinheubach Germany; Study 3: commercial dry feed, IAMS, Coevorden, Netherlands) and had

Table 1
Description of cats in each study.

Study	No. of cats	Cat breed	Cat age (months)	Sex	Body weight (kg)
1	28	DSH	20–23	16M; 12F desexed	3.2–6.0
2	16	ESH	16–30	8M; 8F desexed	3.7–6.6
3	6	DSH	12–36	3M; 3F	2.7–5.0

Abbreviations: DSH, Domestic Shorthair; ESH, European Shorthair; M, male; F, female.

Table 2
Study designs.

Group	No. of treated cats	Dosage	Blood sampling times (hours)[a]
Study 1			
Tigolaner	6	1.5 mg/kg i.v.	(day −5) (3 and 6 min) 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 168, 240, 336, 504, 672, 1008, 1344, 1680, 2016, 2352, 2688, 3024, 3360
Emodepside	6	0.3 mg/kg i.v.	(day −5) (3 and 6 min) 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 168, 240, 336, 504, 672, 1008, 1344, 1680, 2016, 2352
Praziquantel	6	0.2 mg/kg i.v.	(day −5) (3 and 6 min) 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 168, 240, 336, 504, 672, 1008, 1344, 1680, 2016, 2352
Tigolaner, emodepside & praziquantel	10	0.148 ml/kg topical	(day −5) 0.5, 1, 2, 3, 4, 6, 8, 24, 32, 48, 72, 96, 168, 240, 336, 504, 672, 1008, 1344, 1680, 2016, 2352, 2688, 3024, 3193
Study 2			
Tigolaner, emodepside & praziquantel	8	0.37 ml/kg topical	(day −5) 0.5, 1, 2, 3, 4, 6, 8, 24, 32, 48, 72, 96, 168, 240, 336, 504, 672, 1008, 1344, 1680, 2016, 2352, 2688, 3024, 3193
Tigolaner, emodepside & praziquantel	8	0.74 ml/kg topical	(day −5) 0.5, 1, 2, 3, 4, 6, 8, 24, 32, 48, 72, 96, 168, 240, 336, 504, 672, 1008, 1344, 1680, 2016, 2352, 2688, 3024, 3193
Study 3			
Tigolaner, emodepside & praziquantel	6	0.148 ml/kg topical	(pre-dose; 30 min) 1, 2, 4, 8, 24, 48 (day 7, 14, 21, 28)

Note: Treatment day: Day 0.
[a] Values in parentheses represent minutes.

ad libitum access to water.

All cats were individually identified by ear tattoo or electronic transponder. For studies 1 and 2, cats were randomized within sex using a randomized block design, except for some cats which were allocated to group based on their suitability for i.v. administration (Study 1) or to continue existing housing arrangement (Study 2). The studies were not blinded to treatment group.

In Study 1 where i.v. administration was performed, 6 animals per treatment group were treated with a slow manual i.v. bolus using a catheter placed in the *Vv. saphena medialis* or *Vv. saphena lateralis*, and suitable 1-ml single-use syringes. Catheters were placed immediately prior to administration and removed immediately afterwards. Tigolaner (in tetraglycol, C5H9O(OC2H4)nOH, CAS no. 31692-85-0) was administered at 1.5 mg/kg (volume: 70–80 µl), emodepside (in tetraglycol) was administered at 0.3 mg/kg (volume 40–70 µl) and praziquantel (in tetraglycol) was administered at 0.2 mg/kg (volume: 40–60 µl). Low dose rates were administered intravenously to ensure tolerance of an i.v. bolus. Where treatment was administered topically, a spot-on application was manually applied at the base of the head while the hair was divided with 2 fingers. In all studies, topical doses were calculated using individual BWs and the nominal content of the three AIs. The AIs were administered based on the licensed therapeutic dose of 14.5, 3 and 12 mg/kg BW for tigolaner, emodepside and praziquantel, respectively. Cats were restrained for approximately a minute following administration to aid spread of the applied formulation and to prevent any possible run-off. Table 2 provides details of the dosing of each of the three AIs in isolation *via* i.v. administration with topical Felpreva™. Cats were closely observed for 1 h after dosing and at least once daily thereafter.

General health observations (general demeanour, feed consumption, faeces consistency) were performed daily. Specific pre- and post-administration observations were performed before treatment, and 5 h and 29 h after treatment. Physical examinations were performed on study days −7, 14, 28, 39, 53 and 59/60. In studies 1 and 2, BWs were measured on study days −3, 28, 53 and 59/60, whilst in Study 3 BWs were measured on study days 1, 7, 14, 21 and 28. In studies 1 and 2 haematological and clinical biochemistry tests were performed at the beginning and end of the kinetic studies.

Blood samples of ~0.5 ml (Studies 1 and 2) and 1.0 ml (Study 3) were collected into EDTA K2E tubes from the *Vv. cephalica antebrachii* or another suitable vein. Sampling times are shown in Table 2. Plasma was harvested following centrifugation (10 °C, 3220× *g* for 10 min) and subsequently stored frozen at −18 °C.

Urine and faeces were collected from cats in Study 3 for analysis of excretion of the AIs. Sampling days per cat were study days 1, 2, 7, 14, 21 and 28. Cats were kept in stainless steel cages with a litter box for the collection of total urine and faeces. The total volume of urine was determined, thereafter 2 aliquots (A + B) of ~5 ml urine were taken, collected in clear tubes and stored in a freezer set to maintain −18 °C. Faecal samples were weighed and stored in a freezer set to maintain −18 °C.

2.3. Analysis

2.3.1. Pharmacokinetic analytical method

The methods were validated according to "Guidance for Industry: Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration", Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2018 and "European Medicines Agency (EMA): Guideline on Bioanalytical Method Validation" EMEA/CHMP/EWP/192217/2009, 1 February 2012.

A very high specificity resulted from the HPLC separation in combination with MS/MS (tandem mass spectrometry) detection. No signals/peaks interfering with the detection of the analytes were observed in extracts of untreated control samples. Apparent concentrations of all analytes in control samples were below 0.3× limit of quantification (LOQ).

Analysis of all samples was performed after finalisation of the biological part of the study. The plasma samples were deproteinised by mixing 100 µl of plasma with 900 µl of a precipitation mixture of 0.040 g ammonium acetate in 100 ml water plus 0.1 ml formic acid and 600 ml acetonitrile containing the internal standards praziquantel-cyclohexyl-$d_{11}$and [$^{13}C_2H_6$] tigolaner and subsequent centrifugation. Analysis of the AIs tigolaner, emodepside, and praziquantel was conducted by using High Performance Liquid Chromatography with an Agilent Zorbax Eclipse Plus C18 Rapid Resolution, 2.1 × 50 mm, 1.8 µm column, water/formic acid (1000/0.120, v/v) + 10 mMol/l ammonium formate and methanol/formic acid (1000/0.120, v/v) + 10 mMol/l ammonium formate as mobile phase (0–0.5 min at 90/10 v/v, gradient to 0/100 v/v at 3–3.5 min and gradient to 90/10 v/v at 4–5 min) at 60 °C with a flow of 0.6 ml/min. Detection was performed by Tandem Mass Spectrometry (HPLC-MS/MS) using a Sciex API 5500 mass spectrometer in the positive ionisation mode. Quantification of the samples was achieved by use of calibration curves (linear or quadratic, 1/× weighted) obtained by mixed matrix matched standards (containing the internal standards) in the range from 0.07 to 200 µg/l for tigolaner and emodepside and from 0.07 to 100 µg/l for praziquantel. The correlation coefficients were ≥ 0.9985. Recovery (accuracy) of fortified samples was a mean of 100–106% for each of the three AIs with mean relative standard deviation (RSD = precision) between 4.5% and 11.0%. The lower limit of quantification was 1.0 µg/l for tigolaner, 0.2 µg/l for emodepside and 0.1 µg/l for praziquantel in Study 1, whilst in subsequent studies the limit of quantification was 1.0 µg/l for the three AIs.

Urine samples were prepared for analysis by mixing 100 µl urine with 900 µl solvent mixture (as described above), containing labelled tigolaner and praziquantel as internal standards, and then centrifuged. Urine was analysed for the active substances tigolaner, emodepside and praziquantel by HPLC with a YMC Triart Phenyl, 2.1 × 50 mm, 1.9 µm column, water/formic acid (1000/0.120, v/v) + 10 mMol/l ammonium formate and methanol/formic acid (1000/0.120, v/v) + 10 mMol/l ammonium formate as mobile phase (0–0.5 min at 70/30 v/v, gradient to 0/100 v/v at 3–3.5 min and gradient to 70/30 v/v at 4–5 min) at 50 °C with a flow of 0.6 ml/min, and MS/MS detection using a Sciex API 6500 mass spectrometer in the positive ionisation mode. Quantification was performed using matrix-matched standards (including the internal standards for tigolaner and praziquantel) in the range as described above (linear or quadratic, 1/× weighted, correlation coefficients ≥ 0.9991). Recovery, assessed using fortified samples, was a mean of 109% with RSD 4.7% for tigolaner, 109% with RSD of 4.4% for emodepside and 109% with RSD of 5.1% for praziquantel. The lower limit of quantitation was 1 µg/l.

Faecal samples were extracted by mixing 1 or 5 g faeces with 15 or 40 ml acetonitrile containing the internal standards. The mixture was ultrasonicated and shaken by means of an overhead shaker and then centrifuged. The supernatant was transferred into a flask and the residue extracted again with 5 or 30 ml extraction solvent, shaken, and centrifuged. The extracts were combined and filled up to 20 or 100 ml with extraction solvent containing the internal standards, then filtered. The extract was analysed for the active substances tigolaner, emodepside and praziquantel by HPLC-MS/MS using a Sciex API 5500 mass spectrometer under the same conditions as described for urine above. Quantification was performed using matrix-matched standards (including the internal standards for tigolaner and praziquantel) in the range from 0.4 to 75 µg/l, corresponding 8–1500 µg/kg in faecal samples (linear or quadratic, 1/× weighted, correlation coefficients ≥ 0.9995). Recovery, assessed using fortified samples, was a mean of 93% with RSD 5.1% for tigolaner, 95% with RSD of 5.9% for emodepside and 94% with RSD of 7.1% for praziquantel. The limit of quantitation was 10 µg/kg for each analyte.

2.3.2. Pharmacokinetic analysis

PK and statistical evaluations were performed using the standard software Phoenix 64 (WinNonlin®, version 8.1; Pharsight Corporation (a Certara Company), Mountain View, California, USA). Separate

evaluations were performed for the different AIs and study groups. Calculations comprised descriptive statistics on individual concentration data, PK analysis, and descriptive statistics on derived PK parameters (e.g. geometric mean (GM) and geometric standard deviation, minimum and maximum, 95% confidence interval). Plasma concentration-time-profiles were plotted individually and as geometric mean (± geometric standard deviation) curves by AI or study group. Graphic presentation was done using Prism version 8 (GraphPad Software, San Diego, CA, USA).

PK evaluation of the derived plasma concentrations were performed on the observed concentrations and planned sampling times (if actual times did not deviate outside the permitted time window) using non-compartmental methods. Each AI and treatment were evaluated separately. The software calculated a full set of PK parameters automatically.

The software selected the time points used for the terminal phase elimination rate constant calculation (λ_z-calculation) automatically using the "best fit" approach calculated by means of log-linear regression. All parameters were derived from individual animal data sets.

Plasma drug concentrations analysed as being below the quantification limit (< LLoQ) were always entered into the system as "missing". Relative data set weight was always set to 1.

2.3.3. Calculation of topical bioavailability

In Study 1, the plasma exposure of the AIs tigolaner, emodepside and praziquantel were derived for Felpreva® after a single topical treatment at the therapeutic dose rates of 14.5 mg tigolaner, 3.0 mg emodepside and 12.0 mg praziquantel/kg BW. The plasma exposure was compared to the plasma exposure after i.v. dosing using single ingredient reference items and dose rates of 0.1× (tigolaner), 0.667× (emodepside), and 0.03× /0.0167× (praziquantel) the therapeutic dose rates due to the low tolerance of AIs when administered as an i.v. bolus.

The parallel study design did not allow calculating individual bioavailability. The group geometric mean dose normalized total exposure area under the curve extrapolated to infinity (AUCinf/D) was used instead to calculate a mean topical bioavailability/AI (F) using the following equation:

$$F\text{abs} = AUC_{inf}/D, \text{ spot-on}/AUC_{inf}/D, \text{ i.v.}$$

2.3.4. Profiling of the pharmacokinetic characteristics of tigolaner, emodepside and praziquantel

The basic plasma PK characteristics of the active AIs tigolaner, emodepside and praziquantel were profiled after i.v. dosing based on at least the following parameters: Clearance (Cl), V, extrapolated concentration at time of dosing (C0), area under the curve until the last concentration above LoQ (AUC_{last}), AUC_{inf}, dose normalized AUC_{last} and AUC_{inf} (AUC_{last}/D, AUC_{inf}/D), mean residence time (MRT), and half-life. The statistical group mean estimates and suitable statistical parameters describing the distribution (scattering) were provided.

2.3.5. Nonlinear mixed effects model building and evaluation of repetitive dosage of tigolaner

The changes in plasma concentration of tigolaner over time after a single spot-on administration were analysed using the stochastic expectation maximization (SAEM) algorithm implemented in Monolix Suite 2021R2 (Lixoft, Antony, France). We determined the individual values of pharmacokinetic parameters *post-hoc* using the mean of the full posterior distribution. The model was written as described earlier by Sheiner & Ludden (1992) and adopted to veterinary settings (e.g. Pelligand et al., 2016; Wang et al., 2019):

$$y\_ij = F(\varphi\_i, t\_(ij)) + G(\varphi\_i, t\_ij, \beta) \times \varepsilon\_ij$$

$$\varepsilon\_ij \sim N(0, \sigma\text{^}2), \varphi\_i = h(\mu, \eta\_i, \beta\_i)$$

$$\varphi\_i = \mu \times e\text{^}(\eta\_i), \eta\_i \sim N(0, \Omega, \omega\text{^}2)$$

$$j \in \{1, \ldots, \eta\_i\}, i \in \{1, \ldots, N\}$$

where yij is the observed Substance X concentration measured in individual i (N is the number of all individuals) at time tij, whereas j describes the individual sample times from 1 to ni. Function F(φi, tij) is the predicted drug concentration at time tij dependent on the vector of individual pharmacokinetic parameters φi. The term G (φi, tij, β) X εij is the residual error model of F(φi, tij) where εij is an independent random variable distributed in a standard normal distribution with mean 0 and variance σ2. Individual parameters belonging to the vector φi were modelled as a function of the mean population parameter values, μ, individual variability ηi, and individual covariates, βi. The random variable ηi was assumed to be normally distributed with mean value 0, variance-covariance matrix Ω and variance ω2. As a result, individual parameters φi are log-normally distributed. The final model was parametrized with clearance (Cl), volume of distribution (V), absorption rate constant (ka), and lag time (Tlag). Only 4 of 260 (1.5%) concentration-time data points represented values below the limit of quantitation (BLOQ); therefore, a separate handling of BLOQ data was not included in the model.

Model quality was assessed using a set of accepted graphic and numerical tools (Pelligand et al., 2016; Nguyen et al., 2017). Convergence of the SAEM algorithm was checked by inspection of the stability of parameter search and by the precision of parameter estimates. This was measured by the relative standard error (RSE) of the estimate as obtained by the Fisher Information Matrix. The condition number of the eigenvalues was assessed to check for over-parameterization. Standard goodness-of-fit (GOF) plots were used to assess the performances of the different models: individual fits, individual predictions *vs* observations, normalized prediction distribution errors (NPDE), and visual predictive check. Normality and independence of residuals were assessed using histograms, quantile-quantile plots, and autocorrelation of conditional weighted residuals. Normal distribution of the random effects was assessed using the Shapiro-Wilk test as well as by inspection of the full posterior distribution of random effects and residuals. For converging models with satisfactory GOF diagnostics, corrected Bayesian information criterion (BICc) and the precision of the model parameter estimates were used for final model selection. The BICc was selected over the Akaike's Information Criterion (AIC) as it tends to favour more parsimonious models (Mould & Upton, 2013; Wang et al., 2019).

2.3.5.1. Parameter correlation estimates. Visual inspection of η *vs* η values for pharmacokinetic parameter estimates and Pearson's correlation tests were used to evaluate the choice of correlations between the parameters. Correlation of random effects was applied when correlation coefficients were estimated to be high, met the threshold for inclusion ($P < 0.05$) and improved model performance. As recommended by earlier studies (Lavielle & Ribba, 2016; Pelligand et al., 2016), multiple samples from the posterior distribution obtained at the last SAEM iteration were preferred over the empirical Bayes estimates (EBEs) during the evaluation of parameter correlations.

2.3.5.2. Simulation of a multiple-dose administration. After model selection and fit, the R 3.4.4 package *Simulx 3.3.0* (Monolix 2021R2) was used to simulate tigolaner plasma disposition kinetic profiles from final Monolix run files. First, Monolix file was exported to *Simulx* and used to visualize the entire distribution of predicted tigolaner concentration time courses in cats, following a single administration of 14.5 mg/kg as a spot-on. Second, a population with 1000 cats was simulated and a multidose treatment with different intervals between doses (56 days (not shown) and 91 days) and an observation period of 600 days and this population was calculated with the population parameters which were set in the Monolix-file.

2.3.6. Analysis of clinical data

The results of the pre- and post-treatment physical examinations and assessments were evaluated but not statistically analysed. Body weights were summarized as arithmetic mean and standard deviation per measurement and fluctuations in BW during the in-life phase were calculated. Any adverse event observed was described and assessed for a relation to treatment. Haematology and clinical chemistry data were analysed by means of Advia® 120 Haematology System (Bayer Diagnostics, Tarrytown, USA). Clinical chemistry was determined using a reflection photometer (VetTest 8008, IDEXX GmbH, 55286 Wörrstadt, Germany).

3. Results

Key pharmacokinetic parameters of tigolaner, emodepside and praziquantel administered topically as Felpreva® and intravenously alone are presented in Table 3.

3.1. Single dose characteristics

The PK profile of each of the AIs when administered together as Felpreva® spot-on at a dose volume of 0.148 ml/kg showed an initial peak in plasma concentration followed by sustained levels over a prolonged period of time associated with distribution and elimination (Fig. 1). Following the single topical treatment, tigolaner had a calculated plasma exposure (AUC_{inf}) of 1566 mg*h/l, with the peak concentration of 1245 µg/l reached approx. 12 days (297 h) after dosing. Tigolaner was eliminated from plasma with a calculated half-life of almost 24 days (568 h). Emodepside peaked in plasma 37 h after dosing at a concentration of 44 µg/l. Total plasma exposure was 20.60 mg*h/l and calculated half-life was approximately 14 days (348 h). Praziquantel showed a total plasma exposure of 3.69 mg*h/l, with peak concentrations of 47 µg/l reached 5 h after dosing. It was eliminated from plasma at a mean half-life of 9.9 days (237 h) (Tables 4 and 5).

3.2. Bioavailability

Calculated bioavailability following topical application in comparison with i.v. administration was 57% for tigolaner, 90% for emodepside and 48% (first 24 h: 6%) for praziquantel. Pharmacokinetic profiling of the AIs after i.v. dosing was limited to 1.5 mg tigolaner, 0.2 mg emodepside and 0.2 mg praziquantel/kg BW due to limited tolerance of i.v. bolus administration. Pharmacokinetic parameters related to i.v. administration are shown in Table 3. Tigolaner showed a mean plasma exposure (AUC_{inf}) of 300.12 mg*h/kg with a volume of distribution of 4.0 l/kg and clearance of 0.005 l/h/kg. Mean emodepside plasma exposure was 1.61 mg*h/l with a volume of 38.3 l/kg and clearance of 0.131 l/h/kg. Praziquantel showed a plasma exposure of 0.114 mg*h/l and a volume of distribution of 4.95 l/kg and clearance of 1.861 l/h/kg.

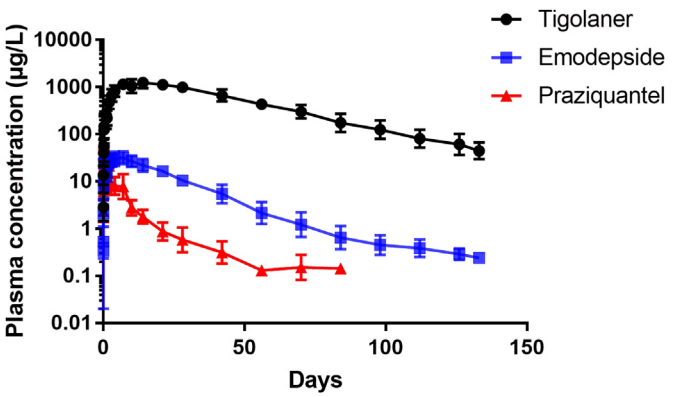

Fig. 1. Mean plasma concentration profiles derived for Felpreva® (in log scale) following a single spot-on administration at the recommended treatment dose (Study 1). Geometric mean and geometric standard deviation of 10 cats treated topically with 0.148 ml/kg that equals to 14.5 mg/kg togolaner, 3 mg/kg emodespide and 12 mg/kg praziquantel.

Table 4
Selected mean plasma pharmacokinetics derived after single dose equivalents of 1×, 2.5× and 5× test item.

Dose level	Dose (mg/kg)	C_{max} (µg/l)	T_{max} (h)	$T_{1/2}$ (h)	AUC_{inf} (mg*h/l)
Tigolaner					
1×	14.5	1245.1	297.0	568.2	1566
2.5×	36.25	2496.9	440.4	569.9	3308
5×	72.5	3574.2	526.6	563.2	5393
Emodepside					
1×	3.0	44.3	36.9	347.5	20.5
2.5×	7.5	71.3	47.8	329.2	36.1
5×	15.0	105.8	61.9	327.6	62.5
Praziquantel					
1×	12.0	47.5	4.8	237.8	3.7
2.5×	30.0	109.8	5.7	210.8	6.8
5×	60.0	176.3	4.7	193.4	14.2

Note: Mean values given as geometric mean.
Abbreviations: T_{max}, time from dosing to the maximum concentration; C_{max}, peak drug plasma concentration; $T_{1/2}$, plasma half-life; AUC_{inf}, area under the concentration *versus* time curve from the time of dosing to infinity (by extrapolation).

3.3. Simulated repetitive administration of tigolaner every 91 days

Based on the kinetic data obtained in Study 1, a profile of repetitive topical administration of tigolaner every 91 days was simulated. As depicted in Fig. 2, there is a roughly a 10% increase of tigolaner concentration in plasma that reaches steady state after the third administration. Overall, the increase in plasma concentration as an indication of cumulation compared to single dose administration (Fig. 1) was modest.

Table 3
Selected mean plasma pharmacokinetics derived for the three active ingredients in cats.

Ingredient	Dose rate mg/kg	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (µg/l)	AUC_{last} (mg*h/l)	AUC_{inf} (mg*h/l)	Cl_pred (l/h/kg)	Vz_pred (l/kg)
Tigolaner topical	14.5	568.2 (26.1)	297.0 (40.5)	1245.1 (35.6)	1516 (32.8)	1566 (32.9)	–	–
Emodepside topical	3.0	347.5 (29.1)	36.9 (215.8)	44.3 (60.9)	20.5 (38.7)	20.6 (38.4)	–	–
Praziquantel topical	12.0	237.8 (23.8)	4.8 (107.7)	47.5 (56.8)	3.6 (19.7)	3.7 (19.6)	–	–
Tigolaner i.v.[a]	1.5	515.4 (54.2)			298 (29.5)	300.1 (29.1)	0.005 (31.7)	4.00 (56.5)
Emodepside i.v.[a]	0.2	202.4 (27.4)			1.5 (80.6)	1.6 (74.4)	0.131 (71.6)	38.25 (89.5)
Praziquantel i.v.[a]	0.2	1.8 (94.8)			0.1 (33.3)	0.1 (32.8)	1.861 (32.2)	4.95 (142.2)

Note: Mean values are given as geometric mean and geometric coefficient of variation in parentheses.
Abbreviations: i.v., intravenously; $T_{1/2}$, plasma half-life; T_{max}, time from dosing to the maximum concentration; C_{max}, peak drug plasma concentration; AUC, area under the concentration *versus* time curve: 0 -Tlast (from the time of dosing to the time to the last quantifiable concentration), 0-inf (from the time of dosing to infinity (by extrapolation)); Cl_pred, systemic clearance; Vz_pred, volume of distribution at steady-state.
[a] Actual mean dose rates applied: 1.62 mg tigolaner, 0.21 mg emodepside, 0.21 mg praziquantel per kg body weight.

Table 5
Selected mean plasma pharmacokinetics and ratios derived after single dose equivalents of 1×, 2.5× and 5× test item.

Dose level	Dose (mg/kg)	C_{max}/D (kg*µg/l/µg) (Geo CV%) [a]	AUClast/D (h*kg*µg/l/µg) (Geo CV%) [a]	Ratio C_{max}/D	Ratio AUClast/D
Tigolaner					
1×	14.5	0.092 (37.9)	103.16 (32.9)	–	–
2.5×	36.25	0.084 (137.8)	108.66 (142.5)	0.91	1.05
5×	72.5	0.053 (34.0)	78.5 (30.7)	0.58	0.76
Emodepside					
1×	3.0	0.015 (61.0)	6.79 (38.7)	–	–
2.5×	7.5	0.009 (71.4)	4.32 (44.2)	0.60	0.64
5×	15.0	0.008 (35.7)	4.5 (36.5)	0.53	0.66
Praziquantel					
1×	12.0	0.004 (57.2)	0.30 (19.9)	–	–
2.5×	30.0	0.003 (51.7)	0.19 (47.7)	0.75	0.63
5×	60.0	0.003 (39.8)	0.25 (22.2)	0.75	0.83

Abbreviations: C_{max}/D, maximum observed concentration divided by dose; AUClast/D, area under the concentration *versus* time curve from the time of dosing to the last measurable concentration divided by the dose.

[a] Mean values given as geometric mean and geometric coefficient of variation in parentheses.

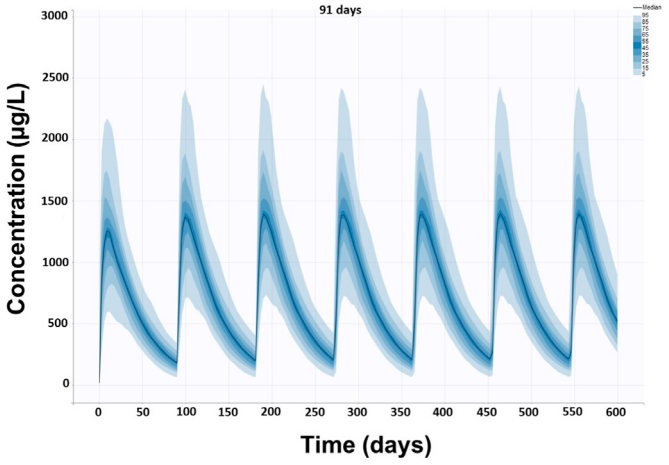

Fig. 2. Simulated profile of repetitive administration of tigolaner every 91 days. Tigolaner data from Study 1 were analysed using the stochastic expectation maximization (SAEM) algorithm. After model selection and fit, tigolaner plasma disposition kinetic profiles were simulated from final Monolix run files. The Monolix file was exported to *Simulx* and used to visualize the entire distribution of predicted tigolaner concentration time courses in cats, following a single administration of 14.5 mg/kg as a spot-on. Second, a population with 1000 cats was simulated and a multidose treatment with different intervals between doses (91 days) and an observation period of 600 days was calculated.

C_{max} increases from *c.*1250 µg/l to 1370 µg/l in steady state. Mean trough values increase from 180 µg/l to 200 µg/l in steady state.

3.4. Dose proportionality

The plasma PKs of the three AIs, tigolaner, emodepside and praziquantel, showed less than proportional increase in rate and extent with increasing dose rates from 1× to 2.5× and 5×, the target dose rate (Tables 4 and 5, Figs. 3 and 4). The less than proportional increase was more obvious for C_{max}/D compared to AUC_{last}/D.

3.5. Excretion

After topical administration, tigolaner was mainly cleared *via* the faeces and approximately 55.5% in males and 53.2% in females of the administered dose was excreted after 28 days. Neglectable amounts of tigolaner was found in urine.

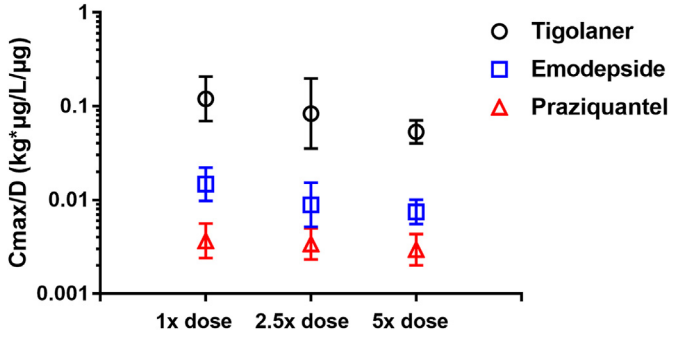

Fig. 3. Extent of plasma exposure (C_{max}/D) at different dose equivalents (data from studies 1 and 2).

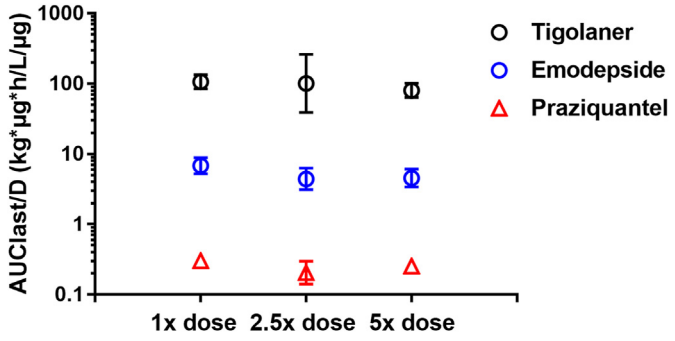

Fig. 4. Rate of plasma exposure (AUC_{last}/D) at different dose equivalents (data from studies 1 and 2).

After topical administration, emodepside was mainly cleared *via* the faeces and approximately 56.7–70.5% of the administered dose was excreted after 28 days.

After topical administration, praziquantel was equally cleared *via* the faeces and *via* the urine for males and showed a slightly higher clearance *via* the faeces compared to the clearance *via* the urine for females. Approximately 1.38–1.47% of the administrated dose was excreted after 28 days.

In conclusion, tigolaner and emodepside seem to be poorly metabolized and mainly excreted *via* the faeces, whereas praziquantel undergoes substantial hepatic metabolism and only less than 2% are excreted equally *via* urine and faeces within 28 days of topical administration.

3.6. Application site and general health

All spot-on treatments were well tolerated by cats. The small volumes and doses used in the i.v. study were well tolerated. Even in the 2.5- and 5 times recommended dose group, none of the cats showed greater abnormalities in haematology, clinical chemistry or physical examination and were considered clinically inconspicuous over the observation period time of 133 days (data not shown).

4. Discussion

The present study reveals the pharmacokinetic profile of tigolaner, together with praziquantel and emodepside after topical administration to cats. The bis-pyrazole tigolaner shares many pharmacodynamic and pharmacokinetic characteristics with the isoxazolines ("laners") including fluralaner, lotilaner, sarolaner and esafoxolaner (Kilp et al., 2016; Geurden et al., 2017; Toutain et al., 2018; Jacquot et al., 2021) that are licensed for cats. Comparable to the isoxazolines, tigolaner has a large volume of distribution, high level of protein binding and slow excretion, which provides it with the persistent characteristics required to protect

cats from an established ectoparasite infestation and subsequent reinfestations. The observed long half-life and high volume of distribution of tigolaner translate into concentrations high enough to offer a three-months protection against fleas and ticks following topical application (Cvejić et al., 2022b; Mencke et al., 2023). As it emerges that fleas are prevalent all year round (ESCCAP, 2022), and for ticks a widespread and longer seasonal activity of *Ixodes ricinus* and *Dermacentor reticulatus* is observed e.g. within Europe, a sustained long-acting protection against ticks and fleas is recommended with the benefit of reduced vector-borne diseases (Bajer et al., 2022).

Felpreva® combines the established Profender® AIs, emodepside and praziquantel with tigolaner. The PK data demonstrate that also emodepside and praziquantel remain available at appropriate levels to exert their antiparasitic activity in the new combination. According to Profender® product information, emodepside reaches maximum serum concentrations of 32.2 ± 23.9 μg/l and praziquantel 61.3 ± 44.1 μg/l. T_{max} for emodepside is 3.2 ± 2.7 days after topical application and 18.7 ± 47 h for praziquantel. Both substances are eliminated from the serum with a half-life of 9.2 ± 3.9 days for emodepside and 4.1 ± 1.5 days for praziquantel (EMA, 2008). These data are in a comparable range as observed in the present study (Table 3) except for an extended half-life for praziquantel (9.9 days *vs* 4.1 days). However, even with a half-life of almost 10 days there is no issue with accumulation of praziquantel. When compared to a different spot-on formulation in cats (Nexgard® Combo, Boehringer Ingelheim, Ingelheim, Germany) the maximally achieved plasma concentrations for praziquantel seem a bit lower (107 ± 59 *vs* 47 ± 56.8 μg/l) but serum half-life seems longer with the formulation tested here (4.3 *vs* 9.9 days). However, the overall range is again similar (Jacquot et al., 2021).

The comparable pharmacokinetic profile of praziquantel and emodepside in Profender® and Felpreva® is reflected by similar clinical efficacy against parasites in naturally infected cats. In a randomized controlled study Felpreva® was proven to be as safe and effective as Profender® in the treatment of intestinal nematode, cestode and lungworm infections in cats under field conditions (Cvejić et al., 2022a), indicating that the pharmacokinetic properties of praziquantel and emodepside released from Felpreva® are reliable and that tigolaner shows only minor interference with absorption, distribution, metabolism, and excretion of praziquantel and emodepside in cats. Although the 2.5- and 5 times recommended dose administrations indicate a slightly less than proportional pharmacokinetic profile, particularly for AUC_{last}/D, almost a linearity can be assumed. Individual cat observations across all treatment groups and for all studies indicate that Felpreva® administered at the recommended treatment dose (RTD) and up to 5× RTD was well tolerated. Due to the relatively long half-life of tigolaner, a simulation of repetitive topical administration (every 91 days) was performed. Although a slight cumulation was noticed, a steady state was reached after the third administration and thereafter for further administrations, the concentration of tigolaner should not increase further. Although the mean concentrations increased slightly from about 1250 μg/l to 1350 μg/l, this is far below concentrations observed at e.g. 2.5× and 5× recommended dose (Table 4) and these higher concentrations still were well tolerated by the cats. Thus, a repetitive administration every 91 days is considered safe.

5. Conclusions

The pharmacokinetic profile of emodepside, praziquantel and tigolaner, the three active ingredients of Felpreva® has been extensively studied. Pharmacokinetic characteristics of the novel ectoparasiticide tigolaner are described for the first time in cats. The large volume of distribution combined with long half-life of tigolaner accounts for its sustained activity against flea and tick infestations for up to three months after a single topical spot-on application with minimal effect on the pharmacokinetic profile of emodepside and praziquantel. Treatment with Felpreva®, including multiples of the recommended treatment dose rate, was well tolerated in cats.

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Ethical approval

The studies were designed in accordance with the standards of Good Clinical Practice (VICH Guideline 9). Cats were handled in compliance with the relevant Animal Care and Use/Ethics Committee approvals. Housing of cats complied with the Directive 2010/63/EU of the European Parliament and of the council of 22nd September 2010 on the protection of animals used for scientific purposes (including Annex III "Requirements for establishments and for the care and accommodation of animals"), the German animal protection act and the German welfare regulation for laboratory animals. Studies were performed in Germany (Studies 1 and 2) and the study design and experimental procedures had been approved by the responsible authorities (LANUV - Regional authority for nature, environment and consumer protection in North Rhine Westphalia). Study 3 was performed in the Netherlands and approved by the Central Authority for Scientific Procedures on Animals (CCD) as required by the Dutch Act on Animal Experimentation.

CRediT authorship contribution statement

Norbert Mencke: Conceptualization, Funding acquisition, Writing – review & editing. **Wolfgang Bäumer:** Formal analysis, Writing – original draft. **Kristine Fraatz:** Investigation, Methodology, Formal analysis, Resources, Supervision. **Ralph Krebber:** Investigation, Methodology, Formal analysis. **Marc Schneider:** Formal analysis, Writing – review & editing. **Katrin Blazejak:** Writing – review & editing. 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: Kristine Fraatz was an employee of Bayer Animal Health GmbH, Germany at the time while the studies reported here were conducted; today, an employee of Elanco Animal Health, Germany. Ralph Krebber is an employee of Bayer AG, Crop Science Division, Germany. Norbert Mencke, Katrin Blazejak and Marc Schneider are employees of Vetoquinol S.A., France.

Data availability

The data supporting the conclusions of this article are included within the article. Raw data generated in the study are confidential.

References

Altreuther, G., Buch, J., Charles, S.D., Davis, W.L., Krieger, K.J., Radeloff, I., 2005. Field evaluation of the efficacy and safety of emodepside/praziquantel spot-on solution against naturally acquired nematode and cestode infections in domestic cats. Parasitol. Res. 97, S58–S64. https://doi.org/10.1007/s00436-005-1445-0.

Bajer, A., Beck, A., Beck, R., Behnke, J.M., Dwużnik-Szarek, D., Eichenberger, R.M., et al., 2022. Babesiosis in southeastern, central and northeastern Europe: an emerging and re-emerging tick-borne disease of humans and animals. Microorganisms 10, 945. https://doi.org/10.3390/microorganisms10050945.

Cvejić, D., Hellmann, K., Petry, G., Ringeisen, H., Hamburg, H., Farkas, R., et al., 2022b. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing emodepside, praziquantel and tigolaner, in treating cats naturally infested with fleas and/or ticks.

Curr. Res. Parasitol. Vector Borne Dis. 2, 100099. https://doi.org/10.1016/j.crpvbd.2022.100099.

Cvejić, D., Mencke, N., Petry, G., Ringeisen, H., Hamburg, H., Hellmann, K., et al., 2022a. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing tigolaner, emodepside and praziquantel, in treating cats with mixed infection with intestinal nematodes, cestodes and/or lungworms. Curr. Res. Parasitol. Vector Borne Dis. 2, 100098. https://doi.org/10.1016/j.crpvbd.2022.100098.

EMA, 2008. Summary of product characteristics for Profender. European Medical Agency. https://www.ema.europa.eu/en/documents/product-information/profender-epar-product-information_en.pdf.

EMA, 2022. Summary of product characteristics for Felpreva. European Medical Agency. https://www.ema.europa.eu/en/documents/product-information/felpreva-epar-product-information_en.pdf.

ESCCAP, 2022. Control of ectoparasites in dogs and cats. European Scientific Counsel Companion Animal Parasites. Guideline 3 Seventh Edition. https://www.esccap.org/uploads/docs/eiw2uedg_0720_ESCCAP_GL3_English_v18_1p.pdf.

Geurden, T., Becskei, C., Farkas, R., Lin, D., Rugg, D., 2017. Efficacy and safety of a new spot-on formulation of selamectin plus sarolaner in the treatment of naturally occurring flea and tick infestations in cats presented as veterinary patients in Europe. Vet. Parasitol. 238 (Suppl. 1), S12–S17. https://doi.org/10.1016/j.vetpar.2012.08.024.

Jacquot, V., Buellet, P., Letendre, L., Tong, W., Li, H., Tielemans, E., 2021. Pharmacokinetics of a novel endectoparasiticide topical formulation for cats, combining esafoxolaner, eprinomectin and praziquantel. Parasite 28, 19.

Kilp, S., Ramirez, D., Allan, M.J., Roepke, R.K., 2016. Comparative pharmacokinetics of fluralaner in dogs and cats following single topical or intravenous administration. Parasites Vectors 9, 296. https://doi.org/10.1186/s13071-016-1564-8.

Lavan, R., Armstrong, R., Normile, D., Vaala, W., 2020. Adherence to veterinary recommendations for ectoparasiticides purchased by cat owners in the USA. Parasites Vectors 13, 541.

Lavan, R.P., Armstrong, R., Newbury, H., Normile, D., Hubinois, C., 2021. Flea and tick treatment satisfaction, preference, and adherence reported by cat owners in the US, UK, or France who treated their cats with transdermal fluralaner. Open Vet. J. 11, 458–467.

Lavielle, M., Ribba, B., 2016. Enhanced method for diagnosing pharmacometric models: random sampling from conditional distributions. Pharmaceutical Res 33, 2979–2988.

Mencke, N., Blazejak, K., Petry, G., Hamburg, H., Ringeisen, H., 2023. Immediate and long-term efficacy of Felpreva®, a new spot-on formulation containing tigolaner, emodepside and praziquantel applied as a single application to cats artificially infested with the cat flea (*Ctenocephalides felis*). Curr. Res. Parasitol. Vector Borne Dis. 3, 100122. https://doi.org/10.1016/j.crpvbd.2023.100122.

Mould, D.R., Upton, R.N., 2013. Basic concepts in population modeling, simulation, and model-based drug development - Part 2: introduction to pharmacokinetic modeling methods. CPT Pharmacometrics Syst. Pharmacol. 2, e38.

Nguyen, T.H., Mouksassi, M.S., Holford, N., Al-Huniti, N., Freedman, I., Hooker, A.C., et al., 2017. Model evaluation of continuous data pharmacometric models: metrics and graphics. CPT Pharmacometrics Syst. Pharmacol. 6, 87–109.

Pelligand, L., Soubret, A., King, J.N., Elliott, J., Mochel, J.P., 2016. Modeling of large pharmacokinetic data using nonlinear mixed-effects: a paradigm shift in veterinary pharmacology. A case study with robenacoxib in cats. CPT Pharmacometrics Syst. Pharmacol. 5, 625–635.

Roeber, F., Jackson, C., Mallett, S., Chambers, M., Smith, V., 2023. Efficacy and safety of Felpreva®, a spot-on formulation for cats containing emodepside, praziquantel, and tigolaner, against experimental infestation with the paralysis tick *Ixodes holocyclus*. Curr. Res. Parasitol. Vector Borne Dis. 3, 100123.

Sheiner, L.B., Ludden, T.M., 1992. Population pharmacokinetics/dynamics. Annu. Rev. Pharmacol. Toxicol. 32, 185–209.

Taweethavonsawat, P., Chungpivat, S., Watanapongchat, S., Traub, R.J., Schaper, R., 2013. Comparative efficacy of a spot-on formulation containing emodepside and praziquantel (Profender®, Bayer) and praziquantel and pyrantel oral tablets (Drontal® for Cats) against experimental *Ancylostoma ceylanicum* infections in cats. Vet. Parasitol. 19, 127–171.

Toutain, C.E., Seewald, W., Jung, M., 2018. Pharmacokinetics of lotilaner following a single oral or intravenous administration in cats. Parasites Vectors 11, 412.

Wang, J., Ren, X., Anirban, S., Wu, X.-W., 2019. Correct filtering for subgraph isomorphism search in compressed vertex-labeled graphs. Inf. Sci. 482, 363–373.

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Efficacy of Felpreva®, a new spot-on formulation containing tigolaner, emodepside and praziquantel, applied as a single application to cats artificially infested with ear mites (*Otodectes cynotis*)

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ABSTRACT

The efficacy of Felpreva® (Vetoquinol), a new spot-on application containing the novel acaricide and insecticide tigolaner in combination with emodepside and praziquantel, was evaluated in cats artificially infested with ear mites (*Otodectes cynotis*). A total of three pivotal dose confirmation studies were conducted, two of them designed as non-interference studies. Cats were artificially infested with *O. cynotis* mites and randomly allocated into groups of 8 cats based on pre-treatment mite counts. Cats were treated once on Day 0, either with Felpreva® (14.5 mg/kg tigolaner, 3 mg/kg emodepside and 12 mg/kg praziquantel) or with placebo. Studies with a non-interference design included two additional groups of cats, treated with Profender® spot-on solution (Vetoquinol) (3 mg/kg emodepside and 12 mg/kg praziquantel) and tigolaner as a mono product (14.5 mg/kg tigolaner). Efficacy was evaluated on Day 28/Day 30 based on total live mite counts after ear flushing. Efficacy was claimed when: (i) at least six control cats per group were adequately infested with mites; (ii) calculated efficacy was $\geq$ 90% based on geometric mean mite counts; and (iii) the difference in mite counts between Felpreva®-treated cats and control cats was statistically significant ($P \leq 0.05$). In two of the three studies, Felpreva®-treated cats were mite-free (100% efficacy) on Day 28/Day 30 and almost full efficacy (99.6%) was seen in the third study. The difference in mite counts between Felpreva®-treated cats and control cats was significant ($P < 0.0001$) in all three studies. All control cats were adequately infested in all three studies. The efficacy of Felpreva® against ear mite (*Otodectes cynotis*) infection in cats was confirmed.

1. Introduction

Otoacariosis caused by the ear mite *Otodectes cynotis* (family Psoroptidae) is frequently found in cats and dogs but does also occur in wild carnivores such as wild cats, foxes, ferrets (Lohse et al., 2002). Ear mites live in the horizontal and vertical ear canal and are occasionally found outside the ear producing pruritic papular skin lesions, often on head, feet and tail tip (Bowman et al., 2002; Curtis, 2004). Off-host, ear mites seem to survive only for a couple of days. Survival times of 12 days were found under natural conditions at temperatures of 12.3–14.2 °C which were linearly declining with increasing temperatures (Otranto et al., 2004). *Otodectes cynotis* are non-borrowing mites that feed on epidermal debris and tissue fluids. The life-cycle is approximately 18–28 days (Bowman et al., 2002; Curtis, 2004) and the entire development (egg,

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larva, nymph and adult) takes place in the ear canal of the host (Muller and Kirk, 2012; Yang and Huang, 2016). Clinical signs in infested animals typically include dark-brown, ceruminous exudate and erythema inside the ear canal in combination with varying degree of pruritus (Yang and Huang, 2016). In cats however, the disease is highly variable, and severity of signs does not necessarily correlate with the number of mites present (Bowman et al., 2002). Affected cats can present anything from apparently healthy (Sotiraki et al., 2001) to severe otitis externa (Yang and Huang, 2016). Ear mite infestations are the most common cause for feline otitis externa, accounting for approximately 50–85% of all clinical cases (Wall and Shearer, 2001; Jacobson, 2002; Brame and Cain, 2021). The disease is highly contagious and affects all types of cats (Noli, 2020). Transmission of *O. cynotis* occurs by direct contact, often from infected mothers to their kittens. Factors like geographical region, age, multi-pet households or frequent access to other cats or hosts can be risk factors (Fanelli et al., 2020). The prevalence of ear mites in cats is variable, though epidemiological data are scarce. In a European multicenter survey, *O. cynotis* was diagnosed in 17.4% of client-owned cats and cats with regular outdoor access had a higher risk for ear mite infestations than cats with only infrequent outdoor access (Beugnet et al., 2014). In a survey from Greece, 14% of kittens and young cats presented to veterinarians were infested with *O. cynotis* (Lefkaditis et al., 2009), whereas in another study, ear mites were found in 25.5% of adult cats (Sotiraki et al., 2001). In stray cats from Portugal, the prevalence was 2.2% (Duarte et al., 2010), whereas the prevalence in shelter cats from Spain was 30% (Fanelli et al., 2020). In a cohort of client-owned, shelter and colony cats from Italy, ear mites were found in 9.8% of the cats (Genchi et al., 2021). When ear mites are diagnosed in an animal, it is generally assumed that all contact animals are infested and treatment recommendations include to treat all susceptible pets in a multi-animal household (CAPC, 2019; ESCCAP, 2022).

Felpreva® is a new broad-spectrum spot-on formulation, which was recently registered for cats in Europe, containing tigolaner, emodepside, and praziquantel at the minimum recommended dose of 14.4 mg/kg, 3 mg/kg, and 12 mg/kg body weight, respectively (EMA, 2021). The product has been demonstrated to have high anthelmintic activity in cats (Cvejić et al., 2022a; Traversa et al., 2022) and high efficacy against common tick and flea species in Europe (Cvejić et al., 2022b), in combination with a fast onset of flea kill (Mencke et al., 2023). The purpose of this article is to present the miticidal activity of the product. Three laboratory studies in cats experimentally infested with *O. cynotis* were conducted. The objective of these studies was to test whether a single topical application of Felpreva® spot-on is effective in eliminating ear mite infestations in cats until four weeks after treatment.

2. Materials and methods

A total of three pivotal dose confirmation studies were conducted, two of them (study #2 and #3) designed as non-interference studies. Two study sites were involved, one was located in the Republic of South Africa (study #1 and #2) and one was located in the USA (study #3). All three studies were in compliance with VICH GL 9 Principles of Good Clinical Practice (EMA, 2000) and internal Standard Operating Procedures (SOPs). The studies were designed following the recommendations of guidelines "Demonstration of efficacy of ectoparasiticides" (EMA, 1994) and "Testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats" (EMA, 2016). All studies were part of a development programme for the regulatory approval of Felpreva®.

2.1. Animals and study design

The studies were randomised, blinded, negative controlled studies, using a parallel group design. Study animals were purpose-bred Domestic Shorthair cats (*Felis catus*) of both sexes, between 8 and 172 months of age and with body weights ranging between 2.2 and 5.9 kg. The cats were housed individually after Day 0, according to accepted local animal welfare regulations (South African National Standard SANS 10386 for study #1 and #2; US Department of Agriculture USDA Animal Welfare Regulations for study #3), and Ethics Committee approvals, where applicable. Cats were fed standard commercial diets appropriate for their age and nutritional needs. Water was supplied *ad libitum*. Food and water were expected to be free of any contaminants that could interfere with the study.

All cats were acclimatised for at least 7 days and were clinically healthy at study start. None of the cats had been treated with any topical or systemic acaricide/insecticide before study inclusion that could have interfered with the study objectives. Approximately 1 month before study inclusion, the cats were artificially infested with live *O. cynotis* mites harvested from donor cats. Success of the artificial infestation was verified by otoscopic examination between Day -6 and Day -2. Live mites were counted in both ears of each cat using a scoring system from 0 (no live mites) to 3 (> 10 live mites) (pre-treatment mite counts).

Cats were blocked within sex on individual pre-treatment mite counts and then randomly allocated to treatment groups. Each study group included 8 cats (males and females) per group. All personnel performing mite count evaluations and involved in general, in clinical and in local tolerance observation procedures were blinded to treatment allocations.

Body weights were determined pre-treatment (between Day -3 and Day -1) for dose calculation purposes and reassessed at study end on Day 28 (study #1 and #2) and Day 30 (study #3). Physical exams were performed pre-treatment (Day -9/Day -2) and at study completion (Day 28/Day 30). General health observations were made daily from Day -7 until study end. Clinical examinations post-treatment were conducted at 1 h (± 15 min), 2 h (± 15 min), 4 h (± 30 min), 6 h (± 1 h) and 8 h (± 30 min), and again on Days 1, 2 and 7. Treatment site evaluations were made shortly before treatment application and again at 1 h (± 15 min), 2 h (± 15 min), 4 h (± 30 min), 6 h (± 30 min), and 8 h (± 30 min) post-treatment, and again on Days 1, 2, 7, 14, 21, and 28.

2.2. Treatment administrations

Dose regimens of all three studies are displayed in Table 1. In study #1, one group of cats was treated with Felpreva® (at the minimum recommended dose of 14.5 mg tigolaner, 3 mg emodepside and 12 mg

Table 1
Design of Felpreva® dose confirmation studies in cats artificially infested with ear mites *Otodectes cynotis*.

Study	Product	Actives and minimum dose rates per kg BW	Dose volume (ml/kg BW)	Ear mite counts
#1	Solketal	na	0.148	Days -3, 14 and 28
	Felpreva®	14.5 mg/kg tigolaner + 3 mg/kg emodepside + 12 mg/kg praziquantel	0.148	
#2	Solketal	na		Days -2, 14 and 28
	Felpreva®	14.5 mg/kg tigolaner + 3 mg/kg emodepside + 12 mg/kg praziquantel	0.148	
	Tigolaner mono	14.5 mg/kg tigolaner	0.148	
	Profender	3 mg/kg emodepside + 12 mg/kg praziquantel	0.148	
#3	Mineral oil	na	0.148	Days -6 and 30
	Felpreva®	14.5 mg/kg tigolaner + 3 mg/kg emodepside + 12 mg/kg praziquantel	0.148	
	Tigolaner mono	14.5 mg/kg tigolaner	0.148	
	Profender	3 mg/kg emodepside + 12 mg/kg praziquantel	0.148	

Abbreviations: BW, body weight; na, not applicable.

praziquantel per kg body weight) and the other group with placebo (solketal, syn. isopropylidineglycerol, a glycerol derivative). In study #2 and #3, groups of cats were assigned to one of the following treatments: (i) Felpreva® (Vetoquinol S.A., France) at the minimum recommended dose; (ii) tigolaner mono spot-on at a dose of 14.5 mg per kg body weight; (iii) Profender® (Vetoquinol S.A., France) at the minimum recommended dose of 3 mg emodepside and 12 mg praziquantel per kg body weight; or (iv) placebo (solketal or mineral oil). The dose volumes were the same for all products including placebo (0.148 ml per kg bodyweight).

Application volumes (calculated as pre-treatment body weight × dose volume per kg body weight) were rounded up to two decimal places. All products were administered once on Day 0, applied as spot-on formulations directly to the skin at the base of skull of each cat.

2.3. Ear mite infestations, ear mite counts and debris/cerumen score

Otodectes cynotis mites used for the experimental infestation procedures were local isolates, one originating from naturally infested cats in South Africa (study #1 and #2) and one originating from naturally infested cats in the USA (study #3). Before study start, mites were harvested from donor cats by lavage with saline solution or by use of cotton swabs. The mites were then deposited into both ear canals of each study cat, either on a tuft of hair or directly. Depending on study site and the experimental model that was used, the infestation dose was at least 80–100 live mites/ear (study #1 and #2) or at least 10 live mites/ear (study #3). Study cats were sedated during the procedure to prevent head shaking and removal of the infestation material from the ears during the first hours after infestation.

Regular qualitative otoscopic examinations were performed pre-treatment on both ears of all cats to verify the infestation success. Cats were eligible for study inclusion when the presence of live ear mites was confirmed; in both ears with at least one ear presenting a minimum of 11 live mites on Day -3 (study #1 and #2) or at least one ear with a minimum of 5 live ear mites on Day -6 (study #3).

Post-treatment mite counts after ear duct flushing were used for the primary efficacy evaluation and performed at study completion on Day 28 (study #1 and #2) or Day 30 (study #3). For the procedure, cats were sedated (medetomidine hydrochloride, Dormitor®, Zoetis, 0.08 ml/kg and ketamine, Anaket-V, Bayer Animal Health, 0.05 ml/kg in study #1 and #2; xylazine and ketamine in study #3) and both ear ducts were filled with Docusol® (5% aqueous solution of docusate sodium, Kyron Laboratories). The ears were massaged lightly to loosen cerumen deposits. Dissolved solution was collected over a 38 μm sieve and the ear canal was flushed repeatedly with warm saline solution until it was considered clean. All collected material from one ear was transferred into a labelled container and microscopically examined. Live mites (larvae, nymphs and adult mites) of both ears were counted, summed up for each animal and recorded as the animal's total ear mite count (quantitative assessment).

In study #1 and #2, additional qualitative assessments were performed on Day 14 and Day 28 which were used for secondary efficacy evaluations. Otoscopic mite counts were made for both ears of each cat using a mite count scoring system of 0 (no live mites), 1 (1–4 live mites), 2 (5–10 live mites), and 3 (> 10 live mites). In addition to mite counts, clinical signs of ear mite infestation were assessed in both ears, using a debris and cerumen score of 0 (no debris/cerumen), 1 (slight debris/cerumen), 2 (moderate debris/cerumen), and 3 (severe debris/cerumen).

2.4. Statistical analysis

Adequacy of infestation was achieved in the placebo (negative control) groups when at least 6 cats were infested with ≥ 11 ear mites (study #1 and #2) or ≥ 10 ear mites (study #3) as a sum of both ears.

The primary efficacy criterion was the efficacy against ear mites. The total number of live ear mite counts on Day 28/Day 30 after ear flushing were used to calculate geometric means (count + 1 data with 1 subsequently subtracted from result). Efficacy (%) was calculated using the Abbott formula: 100 × (C – T)/C, where C is the geometric mean of live mite counts of cats in the negative control group and T is the geometric mean of live ear mite counts of cats in the treated groups. Group comparisons were made using a one-way analysis of variance (ANOVA) in SAS 9.3 and higher (SAS Institute Inc., Cary, NC, USA), including treatment as a fixed effect. All hypotheses were tested at a two-sided 0.05 level of significance. Efficacy was claimed when efficacy ≥ 90% was calculated and a statistically significant difference ($P \leq 0.05$) between the treatment group and control group was demonstrated. The experimental unit was the individual cat.

Secondary efficacy criteria were evaluated in study #1 and #2, which included otoscopic live mite count reductions and otoscopic improvement of debris/cerumen scores on Day 14 and Day 28. The effect of treatment was assessed for both parameters, by comparing scores (mite count score and debris/cerumen score) on Day 14 and Day 28 with scores at baseline (Day -2/Day -3). Calculations were made using the ear with the higher score of each cat. Differences were assessed using a two-sided, non-parametric test (Cochran-Mantel-Haenszel test) with the 0.05 level of significance.

3. Results

3.1. Efficacy

In all three studies, control cats were adequately infested with ear mites at study end (Day 28/Day 30). The geometric mean mite counts in the negative controls were 111.9 (range 21–881) in study #1, 103.0 (range 15–761) in study #2, and 63.2 (range 11–190) in study #3 (Table 2).

No ear mites were recovered from cats treated with Felpreva® after ear duct flushing at study end in all three studies, except for two cats in study #2 which had 3 and 4 live mites in one ear on Day 28. Efficacy rates were 100% in study #1 and #3; and 99.6% in study #2. The difference of mite counts between Felpreva®-treated cats and control cats was statistically significant ($P < 0.0001$) in all three studies.

Similar results were observed when cats were treated with tigolaner. Efficacy for tigolaner mono spot-on was 99.9% in study #2 (Day 28) and 100% in study #3 (Day 30). A single mite was found in one cat in study #2. All other tigolaner-treated cats were free of ear mites. Mite count reductions in tigolaner-treated cats were statistically significant ($P < 0.0001$).

Efficacy against mites was low when cats were treated with Profender®. Efficacy rates were 31.6% in study #2 and 36.0% in study #3. Mite counts in Profender®-treated cats were not statistically different from mite counts in control cats (study #2).

Comparisons of pre- and post-treatment mite count scores by otoscopic examinations in study #1 and #2 showed that mite count scores of all (100%) Felpreva®- and all (100%) tigolaner-treated cats had improved on Day 14, as well as on Day 28, which was significantly different from control cats on both days and for both studies. The percentage of improved control cats ranged from 37.5% (Day 28) to 50% (Day 14) in study #1 and from 25% (Day 14) to 50% (Day 28) in study #2. The percentage of cats with improved mite count scores after treatment with Profender® (62.5% on both days) was not significantly different compared to control cats (Fig. 1).

Debris and cerumen scores were also improved after treatment with Felpreva®, though less frequently compared to mite count scores. In study #1, 62.5% of Felpreva®-treated cats had improved debris and cerumen scores on both study days whereas in study #2, improved scores were observed in 75% (Day 28) to 87.5% (Day 14) of the cats. When cats were treated with tigolaner alone (study #2), 87.5% of the cats showed improved scores (both days). In comparison, the percentage of improved control cats ranged from 0% (Day 28) to 37.5% (Day 14) in study #1 and from 37.5% (Day 14) to 62.5% (Day 28) in study #2. A significant difference in debris and cerumen scores between Felpreva®-treated cats and

Table 2
Geometric mean mite counts and calculated percent efficacy against ear mites (*Otodectes cynotis*) for treated groups compared to negative controls four weeks after treatment (Day 28/30, 8 cats per group).

Study	Product	Study day	Efficacy (%)	Mean mite counts (geometric mean)	Range	*P*-value
#1	Control group	Day 28	na	111.9	21–881	na
	Felpreva®		100	0	0	<0.0001
#2	Control group	Day 28	na	103.0	15–761	na
	Felpreva®		99.6	0.5	0–4	<0.0001
	Tigolaner mono		99.9	0.1	0–1	<0.0001
	Profender®		31.6	70.4	9–337	0.4621
#3	Control group	Day 30	na	63.2	11–190	na
	Felpreva®		100	0	0	<0.0001
	Tigolaner mono		100	0	0	<0.0001
	Profender®		36.0	40.4	10–550	nd

Abbreviations: nd, not determined; na, not applicable.

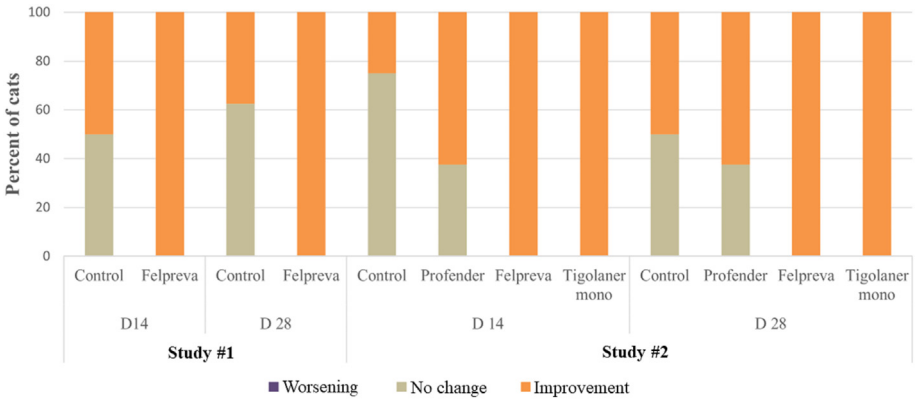

Fig. 1. Changes of post-treatment *Otodectes cynotis* visible live mite count scores in relation to pre-treatment assessed by otoscopic examination (effect of treatment based on worst case score between both ears on Day 14 and Day 28 *versus* pre-treatment (Day -2/Day -3) in comparison to placebo, Cochran-Mantel-Haenszel test, level of significance = 0.05).

control cats was only demonstrated for Day 28 in study #1 and for Day 14 in study #2 (Fig. 2).

3.2. Safety observations

In study #2, six cases of mild erythema at the application site were observed 2 h post-treatment. Cases involved three control cats, two cats treated with Felpreva® and one tigolaner-treated cat. While the location of the erythema indicated a possible product relation, involvement of tigolaner was considered unlikely as the erythema was found in both, tigolaner-treated cats as well as control cats (treated with solketal). The erythema was transient and resolved 4 h post-treatment in all six cats. No other adverse events in relation to treatment were found in this study and no adverse events were reported in study #1 and #3. Overall, the topical application of Felpreva® was well tolerated in cats.

4. Discussion

Results of the three studies demonstrated that treatment with Felpreva® was highly effective in clearing ear mite infestations in cats. A

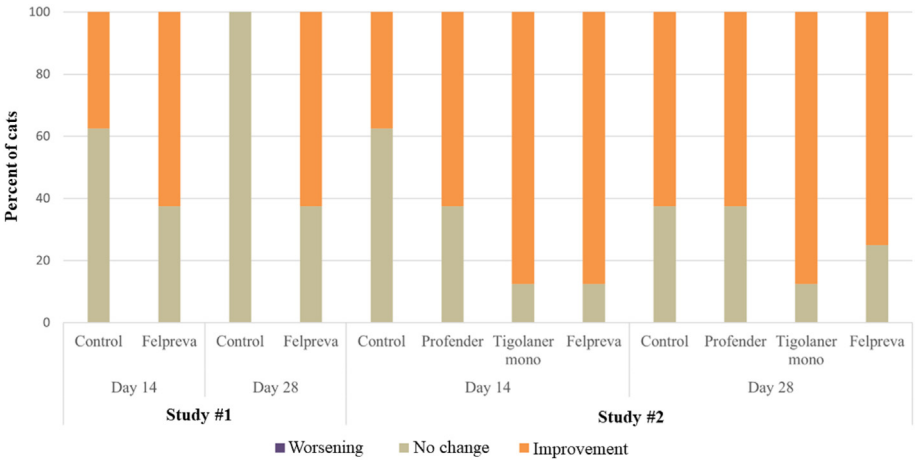

Fig. 2. Changes of post-treatment debris/cerumen scores in relation to pre-treatment assessed by otoscopic examination (effect of treatment based on worst case score between both ears on Day 14 and Day 28 *versus* pre-treatment (Day -2/Day -3) in comparison to placebo, Cochran-Mantel-Haenszel test, level of significance = 0.05).

single topical application at the minimum recommended dose to cats artificially infested with *O. cynotis* provided complete parasitological cure (100% efficacy) in two of three studies and almost full efficacy (99.6%) in the third study, when assessed four weeks after treatment. Ear mites were observed in two cats in the third study, but only low number of mites (3 and 4 live mites, respectively) were recovered after ear duct flushing on Day 28.

In otoscopic examinations on Day 14 (study #1 and #2), all (100%) Felpreva®-treated cats had lower mite counts compared to pre-treatment assessments. This was demonstrated by improved mite count scores which were significantly different from mite count scores seen in control cats. These results indicate that the product's onset of efficacy is fast and that ear mites are rapidly killed inside the ear canal, starting within the first two weeks after treatment. Debris and cerumen scores were also improved on Day 14, though less frequently, as it was observed in only 62.5% (study #1) to 87.5% (study #2) of all Felpreva®-treated cats. This was not unexpected, as it is known that the disappearance of otoacariosis signs may take longer, and that some degree of clinical signs may still be present in cats for several days after testing negative for ear mites (Curtis, 2004).

Both studies with non-interference design (study #2 and #3) confirmed that tigolaner is the miticidal component in the combination product, shown by comparable efficacy results in Felpreva®-treated cats (efficacy of 99.6–100%) and tigolaner-treated cats (efficacy of 99.9–100%) in contrast to Profender®-treated cats (efficacy of 31.6–36.0%).

Tigolaner belongs to the class of bispyrazoles, but its systemic insecticidal and acaricidal activity is similar to that of the isoxazolines. There are three topical isoxazoline products currently marketed for cats, all of them with demonstrated activity against *O. cynotis* mites. Treatment with esafoxolaner, sarolaner or fluralaner provided efficacy rates of 97.2–99.9% for esafoxolaner (in combination with eprinomectin and praziquantel, Nexgard® Combo spot-on for cats, Boehringer-Ingelheim Animal Health; Tielemans et al., 2021), 99.2–99.6% for sarolaner (in combination with selamectin, Stronghold® Plus for cats, Zoetis; Becskei et al., 2017), and 100% for fluralaner (alone or in combination with moxidectin, Bravecto® spot-on for cats, Bravecto® Plus spot-on for cats, MSD Animal Health; Taenzler et al., 2017, 2018), when assessed between Day 28 and Day 30 after treatment. In the Felpreva® studies presented here, efficacy against ear mites was as high as 99.6–100%, suggesting that application of Felpreva® is an equally effective treatment that not only eliminates ear mite infestations from the ear canal but essentially also interrupts the life-cycle of *O. cynotis* mites in almost all cats after only one single application. Ear mites are highly contagious and easily spread in multi-cat and multi-pet environments. Therefore, prevention of disease recurrence will strongly depend on the miticidal activity of a product. Treatment with Felpreva® resulted in high miticidal efficacy without any supportive measures, such as regular cleaning of the ears, as it is often recommended for other miticidal products, especially in-ear products.

Felpreva® is presented as a spot-on solution which is an easy-to-use medicine for a stress-free management of cats. The product was safe and very well tolerated in all three studies. In combination with high ear mite efficacy, these factors are known to have a positive influence on treatment compliance and are generally considered important product characteristics for small animal veterinarians and cat owners.

5. Conclusions

A single spot-on administration of Felpreva® was highly effective in clearing the *O. cynotis* infestations in cats four weeks after treatment. The topical application of Felpreva® was well tolerated in cats.

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Ethical approval

The studies were designed in accordance with the standards of Good Clinical Practice (VICH Guideline 9). Cats were handled in compliance with the relevant Animal Care and Use/Ethics Committee approvals. Cats were housed individually after Day 0, according to accepted local animal welfare regulations (South African National Standard SANS 10386 for study #1 and #2; US Department of Agriculture (USDA) Animal Welfare Regulations for study #3), and Ethics Committee approvals, where applicable.

CRediT authorship contribution statement

Katrin Blazejak: Conceptualization, Funding acquisition, Writing – review & editing. **Alta Viljoen:** Investigation, Methodology, Resources, Data curation. **Reinier Zwiegers:** Formal analysis. **Roland Klopper:** Formal analysis. **Hannah Ringeisen:** Investigation, Methodology, Resources, Supervision. **Gabriele Petry:** Investigation, Methodology, Resources, Supervision. **David R. Young:** Investigation, Methodology, Resources. **Douglas Shane:** Investigation, Methodology, Resources. **Jennifer Spruill:** Investigation, Methodology, Resources, Supervision. **Ronald K. Tessman:** Methodology, Formal analysis, Data curation. **Terry Settje:** Methodology, Formal analysis, Data curation. **Tanja N. Knoppe:** Formal analysis, Writing – original draft, Writing – review & editing. **Norbert Mencke:** Writing – review & editing.

Declaration of competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hannah Ringeisen and Gabriele Petry were employees of Bayer Animal Health GmbH, an Elanco Animal Health Company at the time while the studies reported here were conducted. Katrin Blazejak and Norbert Mencke are employees of Vetoquinol S.A., Paris, France. Tanya N. Knoppe is owner of Vet Advice, Hamburg, Germany. Alta Viljoen is an employee of Clinvet International (Pty) Ltd, Blomfontein, South Africa. Reinier Zwiegers and Roland Klopper are employees of ClinData Blomfontein, South Africa. Jennifer Spruill, Ronald K. Tessman and Terry Settje are employees of Elanco Animal Health, Greenfield, USA. David R. Young was owner of Young Veterinary Research Services, Turlock, CA, USA. Douglas Shane is employee of Young Veterinary Research Services, Turlock, CA, USA.

Data availability

The data supporting the conclusions of this article are included within the article. Raw data generated in the study are confidential.

References

Becskei, C., Reinemeyer, C., King, V.L., Lin, D., Myers, M.R., Vatta, A.F., 2017. Efficacy of a new spot-on formulation of selamectin plus sarolaner in the treatment of *Otodectes cynotis* in cats. Vet. Parasitol. 238 (Suppl. 1), S27–S30.

Beugnet, F., Bourdeau, P., Chalvet-Monfray, K., Cozma, V., Farkas, R., Guillot, J., et al., 2014. Parasites of domestic owned cats in Europe: Co-infestations and risk factors. Parasites Vectors 7, 291.

Bowman, D.D., Hendrix, C.M., Lindsay, D.S., Barr, S.C., 2002. Feline Clinical Parasitology, 1st ed. Blackwell Science, Iowa State University Press, Iowa, USA, pp. 389–394.

Brame, B., Cain, C., 2021. Chronic otitis in cats: Clinical management of primary, predisposing and perpetuating factors. J. Feline Med. Surg. 23, 433–446.

CAPC, 2019. CAPC guidelines. Otodectic mange. Companion Animal Parasite Council. https://capcvet.org/guidelines/otodectic-mite/. (Accessed 8 November 2022).

Curtis, C.F., 2004. Current trends in the treatment of *Sarcoptes*, *Cheyletiella* and *Otodectes* mite infestations in dogs and cats. Vet. Dermatol. 15, 108–114.

Cvejić, D., Hellmann, K., Petry, G., Ringeisen, H., Hamburg, H., Farkas, R., et al., 2022b. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing emodepside, praziquantel and tigolaner, in treating cats naturally infested with fleas and/or ticks. Curr. Res. Parasitol. Vector-Borne Dis. 2, 100099.

Cvejić, D., Mencke, N., Petry, G., Ringeisen, H., Hamburg, H., Hellmann, K., et al., 2022a. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing tigolaner, emodepside and praziquantel, in treating cats with mixed infection with intestinal nematodes, cestodes and/or lungworms. Curr. Res. Parasitol. Vector-Borne Dis. 2, 100098.

Duarte, A., Castro, I., Pereira da Fonseca, I.M., Almeida, V., Madeira de Carvalho, L.M., Meireles, J., et al., 2010. Survey of infectious and parasitic diseases in stray cats at the Lisbon Metropolitan Area, Portugal. J. Feline Med. Surg. 12, 441–446.

EMA, 1994. Demonstration of efficacy of ectoparasiticides. Guideline to Directive 81/852/EEC as amended. European Medicines Agency. https://www.ema.europa.eu/en/documents/scientific-guideline/demonstration-efficacy-ectoparasiticides_en.pdf. (Accessed 8 November 2022).

EMA, 2000. VICH GL9 Guideline on Good Clinical Practices CVMP/VICH/595/98-FINAL https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl9-good-clinical-practices-step-7_en.pdf. (Accessed 8 November 2022).

EMA, 2016. EMEA/CVMP/EWP/005/2000-Rev.3: Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-testing-evaluation-efficacy-antiparasitic-substances-treatment-prevention-tick-flea_en-0.pdf. (Accessed 8 November 2022).

EMA, 2021. EMA/532968/2021. CVMP assessment report for Felpreva® (EMEA/V/C/005464/0000), 9 September 2021. https://www.ema.europa.eu/en/documents/assessment-report/felpreva-epar-public-assessment-report_en.pdf. (Accessed 8 November 2022).

ESCCAP, 2022. Control of ectoparasites in dogs and cats. European Scientific Counsel Companion Animal Parasites ESCCAP Guideline 03, 7th ed., January 2022 https://www.esccap.org/uploads/docs/4ce0ad9k_0720_ESCCAP_GL3_English_v17_1p.pdf. (Accessed 8 November 2022).

Fanelli, A., Doménech, G., Alonso, F., Martínez-Carrasco, F., Tizzani, P., Martínez-Carrasco, C., 2020. *Otodectes cynotis* in urban and peri-urban semi-arid areas: A widespread parasite in the cat population. J. Parasit. Dis. 44, 481–485.

Genchi, M., Vismarra, A., Zanet, S., Morelli, S., Galuppi, R., Cringoli, G., et al., 2021. Prevalence and risk factors associated with cat parasites in Italy: A multicenter study. Parasites Vectors 14, 475.

Jacobson, L.S., 2002. Diagnosis and medical treatment of otitis externa in the dog and cat. J. S. Afr. Vet. Assoc. 73, 162–170.

Lefkaditis, M.A., Koukeri, S.E., Mihalca, A.D., 2009. Prevalence and intensity of *Otodectes cynotis* in kittens from Thessaloniki area, Greece. Vet. Parasitol. 163, 374–375.

Lohse, J., Rinder, H., Gothe, R., Zahler, M., 2002. Validity of species status of the parasitic mite *Otodectes cynotis*. Med. Vet. Entomol. 16, 133–138.

Mencke, N., Blazejak, K., Petry, G., Hamburg, H., Ringeisen, H., Remer, C., et al., 2023. Immediate and long-term efficacy of Felpreva®, a new spot-on formulation containing tigolaner, emodepside and praziquantel applied as a single application to cats artificially infested with the cat flea *Ctenocephalides felis*. Curr. Res. Parasitol. Vector-Borne Dis. 3, 100122.

Muller, G., Kirk, R.R., 2012. Parasitic skin disease. In: Miller, W.H., Griffin, C.E., Campbell, K.L. (Eds.), Muller and Kirk's Small Animal Dermatology, 7th ed. Saunders Elsevier, St. Louis, pp. 304–313.

Noli, C., 2020. Flea biology, allergy and control. In: Noli, C., Colombo, S. (Eds.), Feline Dermatology. Springer Nature Switzerland AG, Cham, Switzerland, pp. 437–449.

Otranto, D., Milillo, P., Mesto, P., De Caprariis, D., Perrucci, S., Capelli, G., 2004. *Otodectes cynotis* (Acari: Psoroptidae): Examination of survival off-the-host under natural and laboratory conditions. Exp. Appl. Acarol. 32, 171–180.

Sotiraki, S.T., Koutinas, A.F., Leontides, L.S., Adamama-Moraitou, K.K., Himonas, C.A., 2001. Factors affecting the frequency of ear canal and face infestation by *Otodectes cynotis* in the cat. Vet. Parasitol. 96, 309–315.

Taenzler, J., de Vos, C., Roepke, R.K., Frénais, R., Heckeroth, A.R., 2017. Efficacy of fluralaner against *Otodectes cynotis* infestations in dogs and cats. Parasites Vectors 10, 30.

Taenzler, J., de Vos, C., Roepke, R.K.A., Heckeroth, A.R., 2018. Efficacy of fluralaner plus moxidectin (Bravecto® Plus spot-on solution for cats) against *Otodectes cynotis* infestations in cats. Parasites Vectors 11, 595.

Tielemans, E., Prullage, J., Tomoko, O., Liebenberg, J., Capári, B., Sotiraki, S., et al., 2021. Efficacy of a novel topical combination of esafoxolaner, eprinomectin and praziquantel against ear mite (*Otodectes cynotis*) infestations in cats. Parasite 28, 26.

Traversa, D., Morelli, S., Di Cesare, A., Strube, C., Raue, K., Bisterfeld, K., et al., 2022. Efficacy of two topical combinations containing emodepside plus praziquantel, and emodepside plus praziquantel plus tigolaner, for the treatment of troglostrongylosis in experimentally infected cats. Curr. Res. Parasitol. Vector-Borne Dis. 2, 100097.

Wall, R., Shearer, D., 2001. Veterinary Ectoparasites: Biology, Pathology and Control, 2nd ed. Blackwell Science, Iowa State University Press.

Yang, C., Huang, H.P., 2016. Evidence-based veterinary dermatology: A review of published studies of treatments for *Otodectes cynotis* (ear mite) infestation in cats. Vet. Dermatol. 27, 221-e56.

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Field efficacy and safety of Felpreva® (tigolaner, emodepside and praziquantel) spot-on for the treatment of natural ear mite infestations (*Otodectes cynotis*) and notoedric mange (*Notoedres cati*) in cats

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ABSTRACT

The miticide efficacy of a single treatment with Felpreva® (tigolaner, emodepside and praziquantel) spot-on solution for cats was evaluated in two European field studies. One study was conducted in cats naturally infested with *Otodectes cynotis*. The other study was conducted in cats naturally infested with *Notoedres cati*. In both studies, the presence of viable mites was confirmed prior to treatment (Day -1/Day 0) and re-evaluated on Day 14 (*O. cynotis* study) and on Day 28 (both studies). Efficacy was calculated based on the number of viable mites found after treatment. In the *O. cynotis* study, the primary criterion was the percentage of mite-free cats after treatment with Felpreva® compared to a sarolaner/selamectin combination (Stronghold® Plus, Zoetis) as a positive control. In the *N. cati* study, the primary criterion was the difference between arithmetic mean mite counts of cats treated with Felpreva® and cats treated with a placebo formulation (solketal). Secondary criteria in both studies were changes in clinical lesion scores after treatment. In both studies, all Felpreva®-treated cats were mite-free (100% parasitological cure) on Day 28, 4 weeks after treatment. Signs of mange on Day 28 were clinically improved in all *O. cynotis*-infested cats (100%) and clinically cured in all *N. cati*-infested cats (100%). There were no records of any adverse events or application site reactions in Felpreva®-treated cats.

1. Introduction

After fleas and ticks, mange mites are probably the most clinically relevant ectoparasites in feline parasitology. Ear mite infestations caused by *Otodectes cynotis* (family Psoroptidae) are common and in privately-owned kittens often found at the age of 3 to 6 months (Lefkaditis et al., 2009). Prevalence in semi-domestic, stray, and shelter cats is variable and can range between 2.2% (Portugal; Duarte et al., 2010) and 30% (Spain; Fanelli et al., 2020). *Otodectes cynotis* are non-borrowing mites that live in the horizontal and vertical ear canal of their host. The clinical picture of otoacariosis typically includes large amounts of dark brown debris inside the ear canal with variable degrees of erythema and pruritus (Miller et al., 2013). Occasionally, ear mites are also found outside the ear, often on the head, feet, and tail tip (Bowman et al., 2002; Curtis, 2004). Infested cats are known to present anything from apparently healthy (Sotiraki et al., 2001) to severe signs (Yang and Huang, 2016). *Otodectes cynotis* mites are the most common cause of feline *otitis externa* (Harvey et al., 2001; Jacobson, 2002; Nuttall, 2020; Brame and Cain, 2021). It is estimated that they account for up to 85% of all *otitis externa* cases in cats (Wall and Shearer, 2001). Ear mites are highly contagious and not very host-specific, thus often seen in multi-cat/multi-pet household situations (Nuttall, 2020).

Notoedric mange (feline scabies) caused by *Notoedres cati* (family Sarcoptidae) is generally considered a rare disease in cats (Wall and Shearer, 2001), though it is known to appear in epizootics (Miller et al., 2013). Cats living in colonies, breeding facilities, or catteries are therefore predisposed (Leone and Han, 2020). Actual prevalence data are scarce. In two studies on stray cats, the prevalence of *N. cati* ranged between 0.6% in Israel (Salant et al., 2014) and 2.35% in Greece (Lefkaditis et al., 2015). It is a highly contagious disease that progressively

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affects the cat's health and can be fatal if left untreated (Deplazes et al., 2021). *Notoedres cati* are burrowing mites that live in tunnels in the stratum corneum of the epidermis. Clinical signs in infested cats are pruritus, papules, thick crusts, thickened skin, and alopecia. Signs characteristically start at the margins of the pinna of the ear and rapidly spread to the whole ear, face, eyelids, and neck. Self-grooming and sleeping in a curled position may extend lesions to the feet and perineum of the cat. Pruritus can be intense, and lesions caused by self-trauma are often observed, which increases the risk for secondary bacterial or yeast infections (Miller et al., 2013; Leone and Han, 2020). If not treated, cats may develop lethargy, dehydration, and weight loss. Death is rare but can occur and is more frequently seen in young kittens and immunosuppressed cats (Bowman et al., 2002; Foley et al., 2016; Leone and Han, 2020).

Felpreva® (Vetoquinol S.A. Lure, France) is a new long-acting spot-on solution for cats using a fixed combination of tigolaner, emodepside and praziquantel. The product was registered in the European Union (EMA, 2021) and possesses broad-spectrum activity against both, endo- and ectoparasites. Previous reports have described the high anthelmintic efficacy of Felpreva® in cats naturally infected with intestinal nematodes, cestodes, and lungworms (Cvejić et al., 2022a; Traversa et al., 2022). Moreover, ectoparasite studies demonstrated a 3-month efficacy in cats naturally infested with ticks and fleas (Cvejić et al., 2022b), a fast onset of flea efficacy (Mencke et al., 2023) and high efficacy in cats infested with the paralysis tick *Ixodes holocyclus* (Roeber et al., 2023). More recently, Felpreva® was reported to be highly active against artificial infestations with *O. cynotis* mites (Blazejak et al., 2023). This article aims to extend recent work by presenting the miticidal efficacy in cats naturally infested with *O. cynotis* and *N. cati* mites. Efficacy was assessed in two European field studies. The objective of the two studies reported here was to assess whether a single treatment with Felpreva® is highly effective in eliminating natural infestations with both mange mite species 4 weeks after treatment.

2. Materials and methods

Two field studies were conducted, one in cats naturally infested with *O. cynotis* (Study 1) and one in cats naturally infested with *N. cati* (Study 2). Cats with *O. cynotis* infestations were enrolled in 15 different study sites located in Hungary and Portugal. Cats with *N. cati* infestations were enrolled in one study site in Albania.

Both studies were in compliance with the principles of Good Clinical Practice (EMA, 2000) and followed the recommendations of the guideline "Demonstration of efficacy of ectoparasiticides" (EMA, 1994). The studies were part of the development programme for the regulatory approval of Felpreva®.

2.1. Animals and study design

Cats with clinical signs of otodectic (Study 1) or notoedric (Study 2) mange were eligible for study inclusion when the presence of viable mites was confirmed pre-treatment.

2.1.1. Study 1: Otodectes cynotis

The study in *O. cynotis* infested cats was a positive controlled, blinded, randomised, multicenter and multiregional field study with seven participating veterinary clinics in Portugal and eight veterinary clinics in Hungary. All cats enrolled in the study were client-owned cats. Eligible households had a maximum of five animals (a maximum of three cats and two dogs). One cat per household was nominated as the primary patient for the efficacy and safety evaluations. Other cats of the same household were classified as supplementary cats. Supplementary cats received the same treatment as the primary cat and were monitored for safety, but not included in the efficacy evaluations. Dogs living in the same household were treated against ear mites but were not included in any efficacy or safety evaluation of the study.

All enrolled cats (primary and supplementary) were clinically healthy on Day 0 (except for confirmed mite infestation), non-pregnant, non-lactating, and not treated with any ectoparasiticide with known miticidal efficacy within the last 3 months prior to Day 0. Cats had to be at least 10 weeks-old with a minimum body weight of 1.25 kg.

Physical exams, body weights, and assessment of the application site were taken prior to treatment on Day 0, and again on Day 14 (± 2) and Day 28 (± 2). The presence or absence of ear mites and clinical signs of ear mite infestation. For detailed information on the clinical assessment refer to Table 1. Ear mite lesions were assessed for both ears of each cat on Day 0 prior to treatment, and again on Day 14 (± 2) and Day 28 (± 2). Ears were not cleaned after otoscopic examinations.

Grooming and bathing of the cats was reduced to a minimum during the study and specifically not permitted within 48 h after treatment and 48 h before a scheduled visit.

Blinding was ensured by the separation of study roles. Treatments on Day 0 were applied by trained personnel (dispensers) not involved in diagnosing viable ear mite infestations, assessment of ear mite lesions, or any other clinical observations. All personnel (veterinarians) responsible for the diagnosis and assessment of mite infestations and lesion scores were blinded to treatment allocations. Animal owners were also unaware of treatment allocations.

2.1.2. Study 2: Notoedres cati

The study in *N. cati*-infested cats was a randomized, blinded, negative-controlled, parallel-group, single-center study which was conducted in Albania. Cats enrolled in this study were client-owned, naturally infested cats. For the duration of the study, all cats were housed individually in a controlled study facility. Cats were admitted

Table 1
Ear lesion assessment in *Otodectes cynotis*-infested cats by use of *Otodectes*-induced ear lesions (OEL) scores. Criteria for the analyses of post-treatment *versus* pre-treatment OEL scores to determine the treatment effect.

Clinical signs	*Otodectes*-induced ear lesions (OEL) scoring			
	Absent (0)	Mild (1)	Moderate (2)	Severe (3)
Head shaking; Pruritus – ear scratching; Trauma or alopecia of the pinna; Ulceration of the ear canals; Debris in the ear canals	Absent	Low intensity/density, covering a small area	Great intensity/density over a small area OR Medium intensity/density affecting a large area	Great intensity/density covering a large area

Notes: OEL scores (= sum of scores with values of 0–18) calculated for both ears of each cat on Day 0, Day 14, and Day 28. The ear with the higher OEL score was used for post-treatment *versus* pre-treatment comparisons. Treatment effect = percentage of cats with improved, worsened, and no change in OEL scores in the respective study period (Day 0-Day 14; Day 0-Day 28). Improved: maximum score on Day 14/Day 28 < maximum score on Day 0. Unchanged: maximum score on Day 14/Day 28 = maximum score on Day 0. Worsened: maximum score on Day 14/Day 28 > maximum score on Day 0.

without any acclimatization period. During the study, cats were maintained on their usual feed and water routine and observed daily for general health. After study completion on Day 28, all cats were returned to their animal owners. Ownership of each cat always remained with their respective owner for the entire study duration.

Cats were clinically healthy on Day 0 (except for confirmed mite infestation), non-pregnant, non-lactating, and not intended for breeding for a total of 4 months following administration of the study treatments. None of the cats had been treated with an ectoparasiticide with known miticidal efficacy within the last 3 months prior to Day 0. Cats younger than 10 weeks and weighing less than 1 kg were not eligible for enrolment.

Physical examinations were performed pre-treatment on Day -1 (+1) and Day 0 and again on Day 14 and Day 28. Body weights were measured on Day -1 (+1) and Day 28. Assessments of the application site were made on Day -1 (+1) and on Day 0 prior to treatment, 4 and 8 h after treatment, and again on Days 1, 2, 7, 14, 21, and 28. Assessments for the presence of live mites were performed on Day -1 (+1) and on Day 28. Clinical signs of notoedric mange were assessed on Day 0, Day 14, and Day 28. For detailed information on the clinical assessment refer to Table 2.

Blinding was ensured by the separation of study roles. Treatments on Day 0 (+1) were applied by personnel not involved in diagnosing viable mite infestations, assessment of notoedric lesions, or any other clinical observations. All personnel responsible for the diagnosis and assessment of mite infestation and lesion scores were blinded to treatment allocations. Animal owners were also unaware of treatment allocations.

2.2. Randomization and treatment administrations

2.2.1. Study 1: Otodectes cynotis

Eligible cats were randomized per household in the sequence of inclusion and assigned to one of two treatment groups. Allocations were made using a block design and a 1:1 treatment ratio. Cats were treated topically with a spot-on formulation once on Day 0, either with Felpreva® (Vetoquinol Lure, France) or with Stronghold® Plus (Zoetis Belgium SA). All cats from the same household (primary and supplementary cats) were allocated to the same treatment group. Treatment administration was the responsibility of the assigned study dispenser in each clinic. The appropriate pipette size was selected based on the cat's pre-treatment body weight, to provide a minimum recommended dose rate of 14.4 mg tigolaner, 3 mg emodepside, and 12 mg praziquantel per kg body weight for Felpreva® and a minimum of 6 mg selamectin and 1 mg sarolaner per kg body weight for Stronghold® Plus. Both products were applied according to label instructions directly to the skin at the base of the skull. When dogs were present in the household, these were treated with a marketed oral miticidal product (Bravecto® chewable tablets for dogs, Merck Animal Health).

Table 2
Skin lesion assessments in *Notoedres cati*-infested cats by use of *Notoedres*-induced skin lesion (NISL) scores. Criteria for the analyses of post-treatment *versus* pre-treatment NISL scores to determine the treatment effect.

	Notoedres-induced skin lesions (NISL) scoring			
	Absent (0)	Mild (1)	Moderate (2)	Severe (3)
Severity	No signs of skin lesions, alopecia and scratching	Mild skin lesions, mild alopecia, occasional scratching	Moderate skin lesions, moderate alopecia, intensive scratching, scratch wounds	Severe skin lesions, severe alopecia, thick/crusty and scabby appearance of the skin, intensive scratching, scratch wounds
Extension	No skin lesions	< 50% of body skin surface	≥ 50% of body skin surface	na

Notes: NILS scores (= sum of scores with values of 0–5) calculated for each cat on Day -1 and Day 28. Treatment effect = percentage of cats classified as clinically cured, clinically improved, or clinical failure on Day 28 in comparison to Day -1. Clinical cure: NISL score = 0 on Day 28. Clinical improvement: NISL score < 50% of NISL score on Day -1. Clinical failure: NISL score ≥ 50% of NISL score on Day -1.
Abbreviation: na, not applicable.

2.2.2. Study 2: Notoedres cati

On Day 0, eligible cats were randomized to treatment groups based on pre-treatment mite counts. Cats were blocked into two groups of cats, one group with > 10 mites/cat and one group with ≤ 10 mites/cat. Within each block, cats were then randomly allocated to Felpreva® or placebo (solketal syn. isopropylidineglycerol, a glycerol derivative) in a 1:1 treatment ratio. Study treatments were applied once on Day 0.

Cats allocated to Felpreva® were treated at the minimum recommended dose rate of 14.4 mg tigolaner, 3 mg emodepside, and 12 mg praziquantel per kg body weight. Cats allocated to placebo received solketal. Dose volumes per kg body weight were the same for both products (0.148 ml/kg body weight). Application volumes were calculated (pre-treatment body weight × dose volume per kg body weight, rounded up to two decimal places) and administered once on Day 0 directly to the skin at the base of the skull of each cat.

2.3. Efficacy assessments

2.3.1. Study 1: Otodectes cynotis

2.3.1.1. Presence of Otodectes cynotis: mite counts. Otoscopic examination and/or microscopic examination of aural canal debris and exudates were used to confirm the presence or absence of live *O. cynotis* mites (immature and adult stages) in each primary cat. The presence/absence of ear mites was assessed on Day 0 (prior to treatment), on Day 14, and at study completion on Day 28. Mite counts were performed once on Day 0 prior to treatment to ensure that all eligible cats were adequately infested (minimum of 3 live ear mites present in at least one ear).

2.3.1.2. Clinical signs of Otodectes cynotis infestation: Otodectes-induced ear lesion (OEL) score. Clinical signs of ear mite infestation were assessed for both ears of each cat on Day 0 prior to treatment, and again on Day 14 and Day 28. Assessments were made using the OEL score. Each cat was assessed for head shaking, pruritus (ear scratching), trauma or alopecia at the pinna, erythema, and debris in the ear canal using a scoring system of 0 (absent), 1 (mild), 2 (moderate), or 3 (severe). The sum of all scores for one ear was the OEL score. The ear with the higher OEL score of each cat was used for the efficacy evaluations (Table 1).

2.3.2. Study 2: Notoedres cati

2.3.2.1. Presence of Notoedres cati: mite counts. Deep skin scrapings on Day -1 and on Day 28 were used to confirm the presence or absence of viable *N. cati* mites in each cat. Samples from an area of approximately 1 cm^2 were collected from three different body sites suspected of being mite-infested and examined microscopically. Viable larvae, nymphs, and adult mites of all three scrapings were counted and results were summed up to a total number of viable mites. All enrolled cats were mite-positive on Day -1.

2.3.2.2. Clinical signs of Notoedres cati infestation: Notoedres-induced skin lesions (NISL) score. Clinical signs of notoedric mange were evaluated on Days -1, Day 14, and Day 28, just before any skin scrapings were taken. The severity of notoedric skin lesions was determined using a scoring system of 0 (no lesions, no alopecia, no scratching) to 3 (severe

skin lesions, severe alopecia, intensive scratching). The extent of notoedric skin lesions was determined using a score from 0 (no skin lesions) to 2 (≥ 50% of the body skin surface involved; Hellmann et al., 2013). The sum of both scores (severity and extent) was the NISL score which was used for the efficacy evaluations (Table 2). All enrolled cats had a minimum NISL score of 1 on Day -1.

2.4. Safety assessments

In both studies, all enrolled cats (including supplementary cats of Study 1) were regularly assessed for safety within scheduled or when needed unscheduled study visits. Any sign of abnormal health and any sign at the application site were documented for each cat either observed by the veterinarian (both studies) or reported by the animal owner (Study 1).

The application site was assessed in Study 1 on Day 0 (before treatment), Day 14, and at study completion on Day 28. In Study 2, assessments were made on Day 0 (before treatment and 4 and 8 h after treatment) and on Days 1, 2, 7, 14, 21, and 28.

2.5. Statistical analysis

All calculations were made in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The experimental unit was the individual (primary) cat in both studies.

2.5.1. Study 1: Otodectes cynotis

2.5.1.1. Efficacy analyses. Efficacy analyses included data of all primary cats that completed the study per protocol (per protocol population). The presence or absence of live ear mites was summarised by treatment group and study day. The parasitological cure rate, defined as the percentage of mite-free cats in respective treatment group (Felpreva® or Stronghold® Plus) was calculated for Day 14 (secondary efficacy criterion) and Day 28 (primary efficacy criterion). Non-inferiority of the parasitological cure rate for Felpreva®-treated cats compared to Stronghold® Plus-treated cats was assessed for Day 14 (secondary criterion) and Day 28 (primary criterion) using a generalised linear mixed model with fixed treatment effects and random clinic effects. The test was one-sided with a significance level of 2.5%. Non-inferiority was demonstrated if the lower limit of the 97.5% confidence interval (CI) of the difference in efficacy between both products was greater than -15%.

The effect of treatment on OEL scores (secondary criterion) was compared between both treatment groups with the Cochran-Mantel-Haenszel test, stratified by clinic (reported as a risk ratio with a two-sided 95% confidence interval and 5% level of significance). Both ears of each cat were scored on each observation day (Days 0, 14, and 28) to identify the ear with the higher score which was then used for treatment effect comparisons. The treatment effect was calculated as the percentage of animals with improved, worsened, and with no change in OEL scores in the respective study period (Day 0-Day 14; Day 0-Day 28, Table 1).

2.5.1.2. Safety analyses. Safety analyses included data for all primary and supplementary cats (intention to treat population). The percentage of adverse events (non-serious and serious) and the percentage of suspected adverse drug reactions were compared between both treatment groups with a Fisher's exact test (two-sided 95% confidence interval, 5% level of significance).

2.5.2. Study 2: Notoedres cati

2.5.2.1. Efficacy analyses. Efficacy analyses included data for all cats that completed the study per protocol (per protocol population). The total number of viable mite counts on Day 28 was summarised by treatment group. The primary efficacy criterion was the difference in arithmetic mean mite counts between cats in the Felpreva® group and cats in the placebo group. Efficacy (%) was calculated using the Abbott formula: $100 \times (C - T)/C$, where C is the arithmetic mean of viable mite counts of cats in the placebo group and T is the arithmetic mean of viable mite counts of cats in the Felpreva® group. Group comparisons were made with a test for superiority by applying the one-sided Wilcoxon-Mann-Whitney test with a Mann-Whitney (MW) measure of 0.50 (equality) as a traditional benchmark.

The treatment effect on NISL scores (secondary criterion) was compared between both treatment groups with the Mantel-Haenszel Chi-square statistic (two-sided 95% confidence interval, 5% level of significance), calculated as the percentage of animals classified as clinically cured, clinically improved or clinical failure on Day 28 compared to Day -1 (Table 2).

Table 3
Animal characteristics at the study inclusion of cats naturally infested with *Otodectes cynotis* (Study 1) and *Notoedres cati* (Study 2).

	Study 1: *O. cynotis*-infested cats ($N = 148$)		Study 2: *Notoedres cati*-infested cats ($N = 20$)	
	Felpreva® ($n = 78$)	Stronghold® Plus ($n = 70$)	Felpreva® ($n = 10$)	Solketal ($n = 10$)
Breed				
Pure-bred, *n* (%)	5 (6.4)	6 (8.6)	1 (10.0)	1 (10.0)
Non-pure-bred, *n* (%)	73 (93.6)	64 (91.4)	9 (90.0)	9 (90.0)
Sex				
Female, *n* (%)	44 (56.4)	41 (58.6)	7 (70.0)	4 (40.0)
Male, *n* (%)	34 (43.6)	29 (41.4)	3 (30.0)	6 (60.0)
Age, Range (Mean ± SD, months)	2.5–180 (28.8 ± 38.0)*	2.8–180 (42.7 ± 44.6)*	6–108 (39.0 ± 37.5)	6–60 (23.5 ± 19.5)
Body weight, Range (Mean ± SD, kg)	1.3–7.9 (3.0 ± 1.5)*	1.3–6.3 (3.3 ± 1.2)*	1.0–5.8 (2.9 ± 1.4)	1.1–4.0 (2.7 ± 1.0)
Hair coat length				
Long, *n* (%)	5 (6.4)	6 (8.6)	1 (10.0)	1 (10.0)
Medium, *n* (%)	10 (12.8)	4 (5.7)	0	0
Short, *n* (%)	63 (80.8)	60 (85.7)	9 (90.0)	9 (90.0)
Housing				
Indoors and outdoors, *n* (%)	25 (32.1)	25 (35.7)	7 (70.0)	8 (80.0)
Mostly indoors, *n* (%)	28 (35.9)	18 (25.7)	0	0
Mostly outdoors, *n* (%)	25 (32.1)	27 (38.6)	3 (30.0)	2 (20.0)
Pets in the house				
Single cat, *n* (%)	33 (42.3)	26 (37.1)	na	na
More cats/dogs, *n* (%)	45 (57.7)	44 (62.9)	na	na

Abbreviation: SD, standard deviation; na, not applicable (Cats were individually housed during the study).
Notes: Asterisks indicate statistically significant differences in the average age (Felpreva®-treated cats: 28.8 months; Stronghold® Plus-treated cats: 42.7 months; Wilcoxon test, $P = 0.026$, per protocol population) and body weight (Felpreva®-treated cats: 3.0 kg; Stronghold® Plus-treated cats: 3.3 kg; Wilcoxon test $P = 0.053$, per protocol population).

2.5.2.2. Safety analyses. Safety analyses included data for all cats (intention to treat population). The percentage of adverse events (non-serious and serious), the percentage of suspected adverse drug reactions, and the percentage of application site reactions were compared between both treatment groups with Fisher's exact test (two-sided 95% confidence interval, 5% level of significance).

3. Results

3.1. Comparability of treatment groups pre-treatment

Animal baseline characteristics of both studies are displayed in Table 3. Treatment group comparisons of breed, sex, age, body weight, coat length in the *O. cynotis* study (Study 1) demonstrated statistically significant differences on Day 0 in the average age (Felpreva®-treated cats: 28.8 months, Stronghold® Plus-treated cats: 42.7 months, Wilcoxon test, $P = 0.026$, per protocol population) and a marginally significant differences in the average body weight (Felpreva®-treated cats: 3.0 kg, Stronghold® Plus-treated cats: 3.3 kg, Wilcoxon test $P = 0.053$, per protocol population) on Day 0. These differences, however, were considered not to be clinically relevant, nor with any impact on the statistical endpoint analysis (parasitological cure). The other parameters (breed, sex, coat length) were comparable between both groups. Both treatment groups had similar OEL scores on Day 0 (Felpreva®-treated cats: 7.53; Stronghold® Plus-treated cats: 7.34).

Animal baseline characteristics, NISL scores and mite counts on Day -1 in the *N. cati* study (Study 2) were comparable in both treatment groups (Felpreva®, solketal, per protocol population, data not shown).

3.2. Efficacy Otodectes cynotis study (study 1)

In total, 252 cats (157 primary and 95 supplementary cats) were included in the study. A total of 148 primary cats were treated per protocol and included in the efficacy analyses. Data of all 252 cats were assessed in the safety evaluations.

All 148 primary cats (78 Felpreva®-treated cats and 70 Stronghold® Plus-treated cats) were mite-free (100% efficacy) at study completion on Day 28. A statistical analysis could not be carried out due to missing differences between both treatment groups, but non-inferiority of Felpreva® to Stronghold® Plus was concluded. Efficacy on Day 14 was 89.7% in Felpreva®-treated cats and 88.6% in Stronghold® Plus-treated cats (Table 4). Non-inferiority was demonstrated as the lower limit of the 97.5% CI was greater than the pre-defined -15% (97.5% CI: -0.09).

OEL scores on Day 14 and Day 28 were similar in both treatment groups and no statistical difference was found. Most treated cats had clinically improved by Day 14. A total of 76 out of 78 (97.4%) Felpreva®-treated cats and 68 out of 70 (97.1%) Stronghold® Plus-treated cats showed improved OEL scores on Day 14 (risk ratio: 1.0, 95% CI: 0.95–1.06, $P = 0.869$). On Day 28, improved OEL scores were found in all 78 (100%) Felpreva®-treated cats and in 69 out of 70 (98.6%) Stronghold® Plus-treated cats (risk ratio: 1.01, 95% CI: 0.99–1.04, $P = 0.317$) (Fig. 1). When clinical improvement was displayed as the course of mean OEL scores from Day 0 to Day 28, both treatment groups (Felpreva®/Stronghold® Plus) presented a similar marked decline in mean scores from Day 0 (7.53/7.34) to Day 14 (2.27/2.51), followed by a further though slower decline until Day 28 (0.85/0.87) (Fig. 2).

Table 4
Efficacy of Felpreva® and Stronghold® Plus in the treatment of cats naturally infested with *Otodectes cynotis*, based on the percentage of mite-free cats (parasitological cure) on Day 14 and Day 28 (Study #1, per protocol population).

	Felpreva® ($n = 78$)			Stronghold® Plus ($n = 70$)		
	Parasitological cure[a]	No cure	Efficacy	Parasitological cure[a]	No cure	Efficacy
Day 14	70	8	89.7%[b]	62	8	88.6%
Day 28	78	0	100.0%[c]	70	0	100.0%

[a] Parasitological cure defined as the number of mite-free cats (non-viable *Otodectes cynotis* mites) on the respective study day.
[b] 97.5% confidence limits for the difference: -0.09. Because the lower limit of the 97.5% confidence interval is greater than -0.15, treatment with Felpreva® was non-inferior to treatment with Stronghold® Plus at the one-sided 2.5% significance level.
[c] No statistical analyses were performed due to a lack of differences.

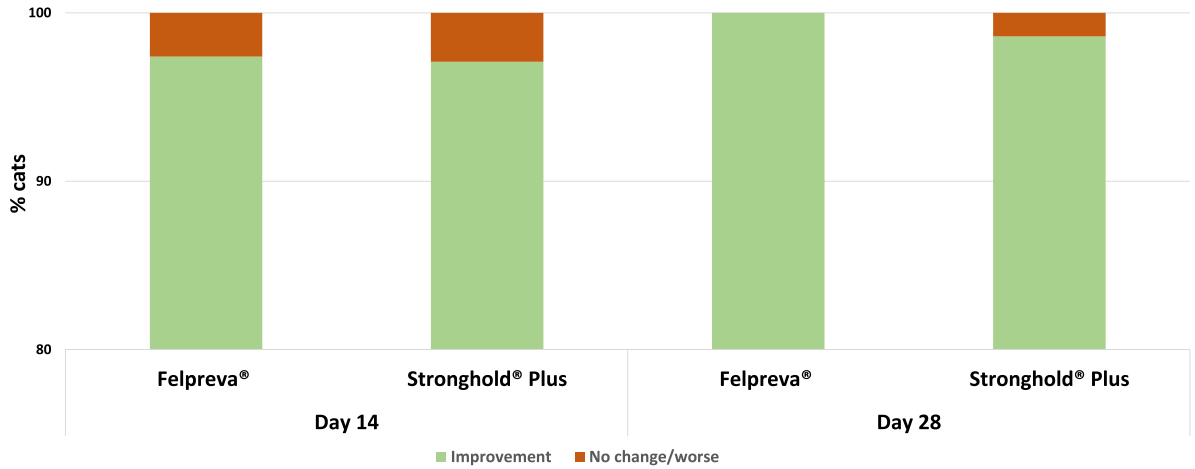

Fig. 1. Changes of *Otodectes*-induced ear lesion (OEL) scores on Day 14 and Day 28 in cats naturally infested with *Otodectes cynotis* after treatment with Felpreva® and Stronghold® Plus (per protocol population). *Note*: Treatment effect = percentage of cats with improved, worsened and with no change in OEL scores in the respective study period (Day 0-Day 14; Day 0-Day 28).

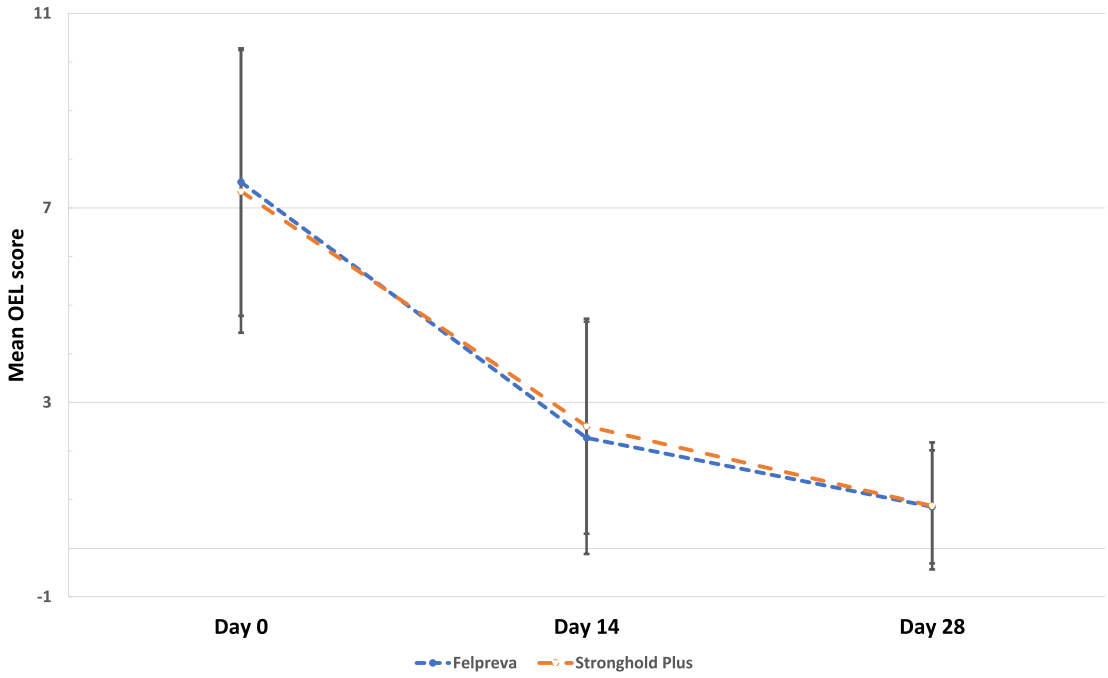

Fig. 2. Course of mean *Otodectes*-induced ear lesion (OEL) scores of Felpreva®- and Stronghold® Plus-treated cats during the study period (Day 0 to Day 28, per protocol population).

3.3. Efficacy Notoedres cati study (study 2)

A total of 20 cats (10 Felpreva®-treated cats and 10 placebo-treated cats) were enrolled in the study. All cats completed the study per protocol and were included in efficacy and safety evaluations.

Four weeks after treatment on Day 28, all Felpreva®-treated cats were mite-free (100% efficacy), whereas an arithmetic mean of 5.5 viable *N. cati* mites was found in placebo-treated cats. Superiority of Felpreva® over placebo was concluded (MW = 1.0, 95% CI: 0.811–1.189, $P \leq 0.0001$) (Table 5).

Clinical signs of notoedric mange (NISL score = 0) were cured in 40% of Felpreva®-treated cats on Day 14 which increased to 100% of the cats on Day 28. In comparison, clinical cure of NISL was not seen in any of the placebo-treated cats, neither on Day 14 nor on Day 28. The difference between Felpreva®-treated cats and placebo-treated cats was statistically significant for both days ($P = 0.029$ for Day 14 and $P < 0.001$ for Day 28) (Fig. 3).

3.4. Safety observations

In both studies, there were no records of any adverse event or application site reaction in Felpreva®-treated cats.

Table 5
Efficacy of Felpreva® *versus* placebo (solketal) in the treatment of cats naturally infested with *Notoedres cati*, based on the differences of total arithmetic mean mite counts on Day 28 (Study 2, per protocol population).

Mite counts	Felpreva® ($n = 10$)	Solketal ($n = 10$)	Felpreva® ($n = 10$)	Solketal ($n = 10$)	Efficacy
	Day -1		Day 28		
Arithmetic mean	5.3	4.2	0	5.5	100%
Standard deviation	2.11	2.70	0	4.35	
Range	2–10	1–11	0	1–14	

Note: Mann-Whitney test, MW = 1.0, 95% confidence interval: 0.811–1.189.

4. Discussion

Results of the two field studies showed that a single treatment with Felpreva® spot-on solution effectively eliminated all *O. cynotis* and all *N. cati* mites in naturally infested cats four weeks after treatment. No adverse reactions were seen in both studies.

The high efficacy of Felpreva® against *O. cynotis* mites presented here is in line with results from earlier dose confirmation studies (Blazejak et al., 2023), where parasitological cure rates in artificially infested cats ranged between 99.6 and 100% four weeks after administration. In this field study, all Felpreva®-treated cats (100%) were free of ear mites on Day 28 and almost 90% of them were already cured by Day 14, demonstrating that *O. cynotis* mites were rapidly and effectively killed after a single application of Felpreva®. It seems likely that the early removal of ear mites from the ear canal had a positive effect on the course of clinical otoacariosis signs suggested by the rapid improvement of post-treatment OEL scores in most of the treated cats. Nearly all (97.4%) of the Felpreva®-treated cats had clinically improved by Day 14 increasing to 100% of the cats on Day 28. These results were achieved without any additional measures or medication other than treating in-contact cats and dogs of the same household. Regular cleaning of the cat's ears, the cat's surroundings, and house cleaning as it has been traditionally recommended for ear mite-infested pets (Harvey et al., 2001; Wall and Shearer, 2001; Curtis, 2004) were not applied in the study.

Treatment with Felpreva® was also highly effective against natural infestations with *N. cati* mites. Four weeks after treatment on Day 28, all Felpreva®-treated cats were mite-free (100% parasitological cure) and all signs of notoedric mange had resolved (100% clinical cure), whereas untreated control cats remained infested (mean of 5.5 viable mites) and did not present any clinical improvement (0% clinical cure; 0% clinical improvement). Traditional treatment protocols for notoedric mange in cats used to be based on the administration of macrocyclic lactones, which must be applied once or twice at 1-month intervals (moxidectin, eprinomectin) or at least twice every two weeks (selamectin, ivermectin; Leone and Han, 2020). Other recommendations include additional weekly lime-sulfur dips or keratolytic shampoos for the treatment of

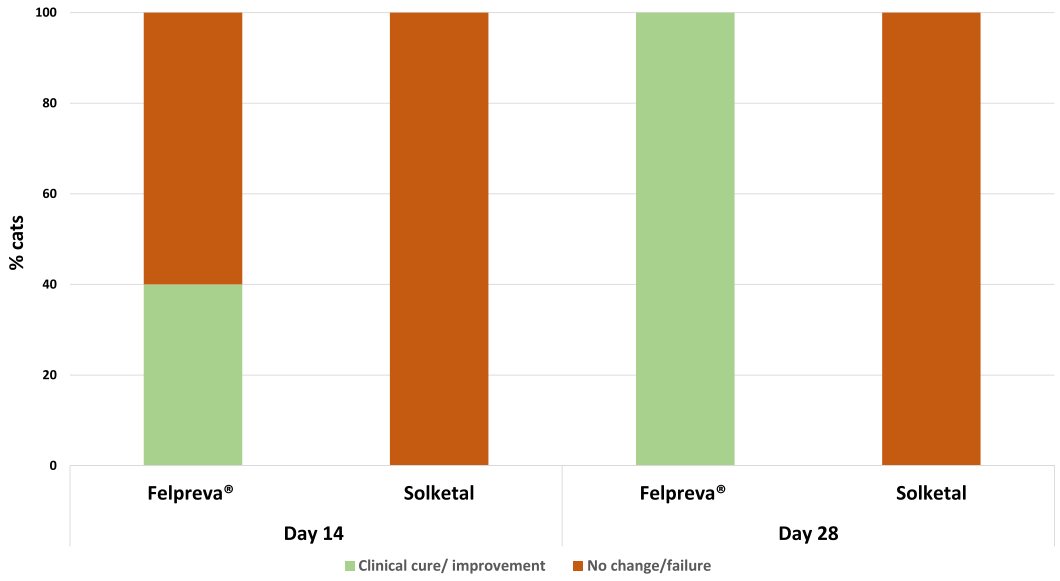

Fig. 3. Changes of *Notoedres*-induced skin lesion (NISL) scores on Day 14 and Day 28 in cats naturally infested with *Notoedres cati* after treatment with Felpreva® and placebo (solketal) (per protocol population). *Notes*: Treatment effect = percentage of cats classified as clinically cured, clinically improved or clinical failure on Day 28 in comparison to Day -1. Clinical cure: NISL score = 0 on Day 28; Improvement: NISL score < 50% of NISL score on Day -1; No change/failure: NISL score ≥ 50% of NISL score on Day -1.

pruritus and to remove skin scales (Schnyder et al., 2019). If necessary, antibiotic and corticosteroid therapy may be applied for severe clinical cases (Bowman et al., 2002). In our study, a complete cure (parasitological and clinical) was achieved in Felpreva®-treated cats within one month after a single treatment and without any further measures such as regular baths or any other concomitant treatment. It is important to note that severe clinical cases of notoedric mange were not seen in our study. Results of our study are based on cats merely displaying mild to moderate signs of notoedric mange (NILS score 1 and 2) on the day of enrolment.

The acaricidal activity of Felpreva® is determined by tigolaner, a novel GABA antagonist which belongs to the class of bispyrazoles. Tigolaner has insecticidal and acaricidal activity, like the class of isoxazolines. In studies evaluating topical isoxazoline products, parasitological cure rates in cats with natural *O. cynotis* infestations ranged between 97.4% (esafoxolaner, Nexgard® Combo, Boehringer-Ingelheim Animal Health; Tielemans et al., 2021) and 100% (fluralaner, Bravecto® spot-on, MSD Animal Health; Bosco et al., 2019) four weeks after treatment. One hundred percent efficacy (based on mite counts) was seen with esafoxolaner in *N. cati*-infested cats on Day 27/28 (Knaus et al., 2021). Results of our studies show that treatment with Felpreva® has equally high efficacy against *O. cynotis* and *N. cati* mites in cats as currently marketed isoxazoline products. In our study, treatment with Felpreva® was statistically non-inferior to a sarolaner/selamectin combination (Stronghold® Plus) when applied to ear mite-infested cats.

This is another report demonstrating the excellent efficacy and safety profile of Felpreva® in cats. In the past, management of otodectic or notoedric mange in cats was laborious and time-consuming and most treatment protocols did not include very feline-friendly procedures. Daily ear cleaning or regular bathing is a traumatic experience for most cats and likely a common reason why pet owners may prematurely cease treatment. The present studies demonstrate that a single treatment with Felpreva® will provide high efficacy against mange mites while offering an easy-to-use medicine with an excellent safety profile for the stress-free management of cats, all characteristics that are likely to enhance owner adherence.

5. Conclusions

A single spot-on administration of Felpreva® was 100% effective in clearing natural *O. cynotis* and *N. cati* infestations in cats four weeks after treatment. Clinical signs of otodectic mange were improved and signs of notoedric mange resolved in all treated cats. The topical application of Felpreva® was very well tolerated by all cats.

Funding

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Ethical approval

The studies were carried out between July and November 2019 (*O. cynotis* study) and between January and March 2020 (*N. cati* study). Clinical field studies are required to confirm the efficacy and safety of a veterinary medicinal product to obtain the marketing authorization according to Directive (2004)/28/EC and 2009/9/EC amending 2001/82/EC in Europe. Both studies were in compliance with the principles of Good Clinical Practice (EMA, 2000) and following the recommendations of the guideline "Demonstration of efficacy of ectoparasiticides" (EMA, 1994). Cat owners agreed to the participation of their animals in the studies prior to enrolment and initiation of treatment, in terms of treatment, mite count and collection procedures, and visits to veterinary practices at the required times. Animal owners gave written consent and allowed their animals to be treated and managed according to the procedures described in the study protocol.

CRediT authorship contribution statement

Katrin Blazejak: Add to CRediT for Hannah Hamburg: Investigation, Methodology, Writing – review & editing. Conceptualization,

Funding acquisition, Writing – original draft, Writing – review & editing. **Dejan Cvejić:** Formal analysis, Data curation, Project administration, Writing – review & editing. **Klaus Hellmann:** Project administration, Supervision, Resources, Writing – review & editing. **Hannah Ringeisen:** Investigation, Methodology, Resources, Supervision, Writing – review & editing. **Hannah Hamburg:** Investigation, Methodology, Resources, Supervision, Writing – review & editing. **Gabriele Petry:** Investigation, Methodology, Resources, Supervision, Writing – review & editing. **Tanja N. Knoppe:** Formal analysis, Writing – original draft, Writing – review & editing. **Norbert Mencke:** Writing – review & editing.

Declaration of competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gabriele Petry, Hannah Ringeisen, and Hannah Hamburg have conducted and funded the studies and are employees of Elanco Animal Health company. Dejan Cvejić and Klaus Hellmann are employees of Klifovet GmbH Munich, Germany. Katrin Blazejak and Norbert Mencke are employees of Vetoquinol, Paris, France. Vetoquinol is the owner of the product Felpreva reported within these studies.

Data availability

The data supporting the conclusions of this article are included within the article. Raw data generated in the study are confidential.

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References

Blazejak, K., Viljoen, A., Zwiegers, R., Klopper, R., Ringeisen, H., Petry, G., et al., 2023. Efficacy of Felpreva®, a new spot-on formulation containing tigolaner, emodepside and praziquantel, applied as a single application to cats artificially infested with ear mites (*Otodectes cynotis*). Curr. Res. Parasitol. Vector Borne Dis. 3, 100122 https://doi.org/10.1016/j.crpvbd.2023.100131.

Bosco, A., Leone, F., Vascone, R., Pennacchio, S., Ciuca, L., Cringoli, G., Rinaldi, L., 2019. Efficacy of fluralaner spot-on solution for the treatment of *Ctenocephalides felis* and *Otodectes cynotis* mixed infestation in naturally infested cats. BMC Vet. Res. 15, 28. https://doi.org/10.1186/s12917-019-1775-2.

Bowman, D.D., Hendrix, C.M., Lindsay, D.S., Barr, S.C., 2002. Feline Clinical Parasitology, 1st ed. Blackwell Science, Iowa State University Press, Iowa, USA, pp. 389–394.

Brame, B., Cain, C., 2021. Chronic otitis in cats: Clinical management of primary, predisposing and perpetuating factors. J. Feline Med. Surg. 23, 433–446. https://doi.org/10.1177/1098612x211007072.

Curtis, C.F., 2004. Current trends in the treatment of *Sarcoptes*, *Cheyletiella* and *Otodectes* mite infestations in dogs and cats. Vet. Dermatol. 15, 108–114. https://doi.org/10.1111/j.1365-3164.2004.00362.x.

Cvejić, D., Hellmann, K., Petry, G., Ringeisen, H., Hamburg, H., Farkas, R., et al., 2022b. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing emodepside, praziquantel and tigolaner, in treating cats naturally infested with fleas and/or ticks. Curr. Res. Parasitol. Vector Borne Dis. 2, 100099 https://doi.org/10.1016/j.crpvbd.2022.100099.

Cvejić, D., Mencke, N., Petry, G., Ringeisen, H., Hamburg, H., Hellmann, K., et al., 2022a. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing tigolaner, emodepside and praziquantel, in treating cats with mixed infection with intestinal nematodes, cestodes and/or lungworms. Curr. Res. Parasitol. Vector Borne Dis. 2, 100098 https://doi.org/10.1016/j.crpvbd.2022.100098.

Deplazes, P., Joachim, A., Mathis, A., Strube, C., Taubert, A., von Samson-Himmelstjerna, G., Zahner, H., 2021. Parasitologie für die Tiermedizin, 4th ed. Thieme, Stuttgart, Germany, pp. 459–466.

Duarte, A., Castro, I., Pereira da Fonseca, I.M., Almeida, V., Madeira de Carvalho, L.M., Meireles, J., et al., 2010. Survey of infectious and parasitic diseases in stray cats at the Lisbon Metropolitan Area, Portugal. J. Feline Med. Surg. 12, 441–446. https://doi.org/10.1016/j.jfms.2009.11.003.

EMA, 1994. Demonstration of efficacy of ectoparasiticides. Guideline to Directive 81/852/EEC as amended. European Medicines Agency. https://www.ema.europa.eu/en/documents/scientific-guideline/demonstration-efficacy-ectoparasiticides_en.pdf. (Accessed 8 November 2022).

EMA, 2000. Guideline on good clinical practices. European Medicines Agency. VICH GL9. CVMP/VICH/595/98-FINAL. https://www.ema.europa.eu/en/vich-gl9-good-clinical-practices-scientific-guideline (Accessed 19 September 2023).

EMA, 2021. CVMP assessment report for Felpreva® (EMEA/V/C/005464/0000). European Medicines Agency 9 September 2021. EMA/532968/2021 https://www.ema.europa.eu/en/documents/assessment-report/felpreva-epar-public-assessment-report_en.pdf. (Accessed 8 November 2022).

Fanelli, A., Doménech, G., Alonso, F., Martínez-Carrasco, F., Tizzani, P., Martínez-Carrasco, C., 2020. *Otodectes cynotis* in urban and peri-urban semi-arid areas: A widespread parasite in the cat population. J. Parasit. Dis. 44, 481–485, 10.1007%2Fs12639-020-01215-7.

Foley, J., Serieys, L.E., Stephenson, N., Riley, S., Foley, C., Jennings, M., et al., 2016. A synthetic review of *Notoedres* species mites and mange. Parasitology 143, 1847–1861. https://doi.org/10.1017/s0031182016001505.

Harvey, G., Harari, J., Delauche, A.J., 2001. Ear diseases of the dog and cat. Manson Publishing Ltd., London, UK, pp. 86–89.

Hellmann, K., Petry, G., Capari, B., Cvejić, D., Krämer, F., 2013. Treatment of naturally *Notoedres cati*-infested cats with a combination of imidacloprid 10%/moxidectin 1% Spot-on (Advocate®/Advantage® Multi, Bayer). Parasitol. Res. 112 (Suppl. 1), 57–66. https://doi.org/10.1007/s00436-013-3281-y.

Jacobson, L.S., 2002. Diagnosis and medical treatment of otitis externa in the dog and cat. J. S. Afr. Vet. Assoc. 73, 162–170. https://doi.org/10.4102/jsava.v73i4.581.

Knaus, M., Capári, B., Szabó, M., Kley, K., Johnson, C., 2021. Efficacy of a novel topical combination of esafoxolaner, eprinomectin and praziquantel against *Notoedres cati* mange in cats. Parasite 28, 27. https://doi.org/10.1051/parasite/2021023.

Lefkaditis, M.A., Koukeri, S.E., Mihalca, A.D., 2009. Prevalence and intensity of *Otodectes cynotis* in kittens from Thessaloniki area, Greece. Vet. Parasitol. 163, 374–375. https://doi.org/10.1016/j.vetpar.2009.04.027.

Lefkaditis, M.A., Sossidou, A.V., Panorias, A.H., Koukeri, S.E., Paştiu, A.I., Athanasiou, L. V., 2015. Urban stray cats infested by ectoparasites with zoonotic potential in Greece. Parasitol. Res. 114, 3931–3934. https://doi.org/10.1007/s00436-015-4688-4.

Leone, F., Han, H.S., 2020. Ectoparasitic diseases. In: Noli, C., Colombo, S. (Eds.), Feline Dermatology. Springer Nature Switzerland AG, Cham, Switzerland, pp. 405–413.

Mencke, N., Blazejak, K., Petry, G., Hamburg, H., Ringeisen, H., Knoppe, T.N., et al., 2023. Immediate and long-term efficacy of Felpreva®, a new spot-on formulation containing tigolaner, emodepside and praziquantel, applied as a single application to cats artificially infested with the cat flea *Ctenocephalides felis*. Curr. Res. Parasitol. Vector-Borne Dis. 3, 100122 https://doi.org/10.1016/j.crpvbd.2023.100122.

Miller, W.H., Griffin, C.E., Campbell, K.L., 2013. Parasitic skin disease. In: Miller, W.H., Griffin, C.E., Campbell, K.L. (Eds.), Muller and Kirk's Small Animal Dermatology, 7th ed. Saunders Elsevier, St. Louis, pp. 298–320.

Nuttall, T., 2020. Otitis. In: Noli, C., Colombo, S. (Eds.), Feline Dermatology. Springer Nature Switzerland AG, Cham, Switzerland, pp. 200–202.

Roeber, F., Jackson, C., Chambers, M., Smith, V., Hume, J., Blazejak, K., Mencke, N., 2023. Efficacy and safety of Felpreva®, a spot-on formulation for cats containing emodepside, praziquantel and tigolaner against experimental infestation with the Australian paralysis tick *Ixodes holocyclus*. Curr. Res. Parasitol. Vector Borne Dis. 4, 100123 https://doi.org/10.1016/j.crpvbd.2023.100123.

Salant, H., Mumcuoglu, K.Y., Baneth, G., 2014. Ectoparasites in urban stray cats in Jerusalem, Israel: Differences in infestation patterns of fleas, ticks and permanent ectoparasites. Med. Vet. Entomol. 28, 314–318. https://doi.org/10.1111/mve.12032.

Schnyder, M., Rehbein, S., Deplazes, P., 2019. Parasitosen. In: Lutz, H., Kohn, B., Forterre, F. (Eds.), Krankheiten der Katze, 6th ed. Thieme, Stuttgart Germany, pp. 468–470.

Sotiraki, S.T., Koutinas, A.F., Leontides, L.S., Adamama-Moraitou, K.K., Himonas, C.A., 2001. Factors affecting the frequency of ear canal and face infestation by *Otodectes cynotis* in the cat. Vet. Parasitol. 96, 309–315. https://doi.org/10.1016/s0304-4017(01)00383-1.

Tielemans, E., Prullage, J., Tomoko, O., Liebenberg, J., Capári, B., Sotiraki, S., et al., 2021. Efficacy of a novel topical combination of esafoxolaner, eprinomectin and praziquantel against ear mite (*Otodectes cynotis*) infestations in cats. Parasite 28, 26. https://doi.org/10.1051/parasite/2021022.

Traversa, D., Morelli, S., Di Cesare, A., Strube, C., Raue, K., Bisterfeld, K., et al., 2022. Efficacy of two topical combinations containing emodepside plus praziquantel, and emodepside plus praziquantel plus tigolaner, for the treatment of troglostrongylosis in experimentally infected cats. Curr. Res. Parasitol. Vector Borne Dis. 2, 100097 https://doi.org/10.1016/j.crpvbd.2022.100097.

Wall, R., Shearer, D., 2001. Veterinary ectoparasites: Biology, pathology and control, 2nd ed. Blackwell Science, Iowa State University Press, p. 262.

Yang, C., Huang, H.P., 2016. Evidence-based veterinary dermatology: A review of published studies of treatments for *Otodectes cynotis* (ear mite) infestation in cats. Vet. Dermatol. 27 https://doi.org/10.1111/vde.12340, 221-e56.