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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 (In press, this issue).

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.