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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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