Comparative Efficacy of Two Fipronil Spot-on Formulations Against Experimental Flea Infestations (*Ctenocephalides felis*) In Dogs

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KEY WORDS: Dog, efficacy, fipronil, spot-on, flea**,** *Ctenocephalides felis*

ABSTRACT

A new fipronil-based spot-on formulation applied once to dogs was evaluated against experimental flea infestations in a parallel group design, randomized, unicentre and blinded controlled study. Eight dogs served as negative controls (group 1), eight dogs were treated with a 10% w/v fipronil-based spot-on solution (Effipro® Spot-on, Virbac SA) at a dosage of 0.67 mL for a dog weighing from 2 to 10 kg and 1.34 mL for a dog weighing from 10.1 to 20 kg (group 2) and eight dogs served as positive controls (group 3) receiving the original fipronil spot-on (Frontline® Top spot, Merial) at a similar dosage. Each dog was infested with 100 unfed adult *Ctenocephalides felis* on days -6, -1, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91. At 48 h after treatment or re-infestation, each dog was combed to remove and count live fleas. Geometric mean flea counts obtained were reduced by 99.7 and 100% in groups 2 and 3, respectively on day 2, compared to the negative control group. Dogs were protected from re-infestations with an efficacy >95 per cent for 93 days in group 2 and for 79 days in group 3. Both 10% w/v fipronil-based spot-ons, despite different vehicles, were equally able to eradicate flea infestation, to prevent new infestations and were well-tolerated.

INTRODUCTION

Flea infestation remains one of the most frequent ectoparasitic conditions of dogs and cats.[1-6] Introduced in 1994, fipronil has been a leader on the flea market products for dogs and cats ever since. First available as a 0.25-percent spray,[7] fipronil was then marketed as a spot-on formulation,[8] and eventually was combined with S-methoprene,[9] also in a spot-on. More recently, pyriprole from the same chemical group had been launched and is available as a spot-on.[10] Finally, as fipronil's patent has recently expired in some countries, new fipronil-based products are now present on the market.

The present study was conducted to evaluate the immediate and sustained ef-

ficacy and the tolerance of a new spot-on formulation (Effipro® Spot-on, Virbac S.A.) with the same qualitative and quantitative composition in terms of active ingredient (fipronil) as the original product (Frontline® Top spot, Merial), but with different vehicles. The efficacy was evaluated against experimental infestations with *Ctenocephalides felis* in dogs. A positive reference control group included dogs treated with the original product.

MATERIALS AND METHODS

Twenty four mongrel dogs (17 female and 7 male) over 4 months of age and weighing from 7.2 to 17.9 kg were initially included in the study. Three dogs (one male and two female) belonging to the negative (untreated) control group were replaced on Day 48 with three female dogs. Dogs were allocated randomly into three groups of eight and each dog was individually housed in a 1.9 x 2.7-meter pen. They were fed a commercial dog diet and water was available *ad libitum*. They were acclimatized for 7 days prior to treatment.

A laboratory bred strain of *Ctenocephalides felis* (ClinVet-2004), routinely fed on cats was used for all infestations. For each experimental infestation, approximately 100 unfed, young adult fleas of mixed sex were placed on each dog's dorsum. The dogs were infested six and one day prior to the treatments and 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91 days after the treatments.

The study was a parallel-arm, randomized block design, unicentre, blinded controlled study. The animals were not treated by an individual involved in the post-treatment assessments and observations. Study groups were coded to blind the staff performing post-treatment assessments and observations. The dogs were ranked within gender in descending order of individual body weight. Within each gender, animals were then allocated to blocks of three dogs each. Within each block, dogs were randomly allocated to groups 1 to 3.

The dogs in group 1 were not treated. The dogs in group 2 were treated with a 10% w/v fipronil-based spot-on solution (Effipro® Spot-on, Virbac SA) at a dosage of 0.67 mL for a dog weighing from 2 to 10 kg and 1.34 mL for a dog weighing from 10.1 to 20 kg. The dogs in group 3 were treated with the original fipronil spot-on (Frontline® Top spot, Merial) at a similar dosage. The solution used in group 2 had the same qualitative and quantitative composition in terms of active ingredient (fipronil) as Frontline® Top spot but some vehicles were different. Both products were applied topically as a single spot on the skin between the shoulders. Care was taken to avoid wetting the hair or applying the dose to an area where the animal could lick it off. Dogs were restrained for one minute after dosing. Concurrent treatments unlikely to interfere with the study were acceptable (antimicrobials, vitamins and mineral supplements and sedatives) and the treatment details were recorded. Substances that may have had an insecticidal or acaricidal activity (e.g. medicated shampoos) were not allowed.

Each animal was submitted to a full clinical examination on days -7, 27 and 63. Additionally on day 48, a full clinical examination was conducted on the three dogs replacing the three dogs withdrawn from the study in the negative control group. Any adverse event was recorded.

Forty-eight hours after the treatment and 48 hours after each challenge, the population of remaining fleas was assessed for each animal. Three operators were involved in the assessment of a specific animal. One person handled and restrained the dog, a second was responsible for combing the dog and a third person was responsible for quantifying the fleas recovered from each comb and recorded the data. During combing, a fine-toothed flea comb was used to recover fleas present in the dog's fur. The method of combing included several strokes of the comb in each area of the animal, each time moving in the same direction, following the pattern of the hair coat. Movement from one part of the dog's fur to the next was via strokes overlapping each other, so that no

area of fur was missed. After completion of the combing procedure for all body areas, the whole procedure was repeated so that all areas were combed twice. All fleas collected were counted and were not replaced on the animals.

Analyses were performed with the commercial software SAS V9.1. For all analyses, the significance threshold was set to $\alpha = 0.05$. The three groups were described and compared before treatment (baseline) on the following criteria: weight, sex, average hair length and flea count. Qualitative parameters were analyzed using a Fisher's exact test and quantitative parameters were analyzed using a Kruskal-Wallis test. Arithmetic and geometric means were calculated for each of the three groups at each time point. For groups 2 and 3, efficacy was calculated at each time point using the mean according to Abbott's formula: Efficacy (%) = 100 x (mean $_{control}$ –mean $_{treated}$)/ mean $_{control}$. Flea count was analyzed by a one-way analysis of variance on ranked data. The p-value of the comparison between groups 2 and 3 was given and compared to 0.017 (Bonferroni correction for multiple comparisons).

RESULTS

The three groups were homogenous at baseline on the following criteria: weight, sex, average hair length and flea count (Table 1). Three dogs from the control group developed severe signs consistent with flea allergy dermatitis (FAD), so they were excluded and replaced with three new dogs on day 48. Two other dogs, one from group 1 and one from group 3 developed similar signs and were excluded on day 79 and not replaced. No adverse events that could be related to the administration of either product were recorded in any of the treatment groups.

The arithmetic mean numbers of fleas that were present in the hair coat of the untreated control dogs and on treated animals 48 hours after each infestation are graphically illustrated in Figure 1. The efficacy, based on geometric means, of both formulations of fipronil is summarized in Table 2.

The results (Figure 1 and Table 2) show that the experimental infestations with *C. felis* were successful, with a mean percentage recovery of *C. felis* on the control dogs 48 hours after each infestation ranging between 51 (day 9) and 88.4 (day 51)

***Figure 1**. Arithmetic mean (sd) Ctenocephalides felis counts 48 hours after treatment with two 10% w/v fipronil-based spot-on solutions on day 0 and 48 hours after weekly re-infestation with 100 unfed adult fleas over 13 weeks.*

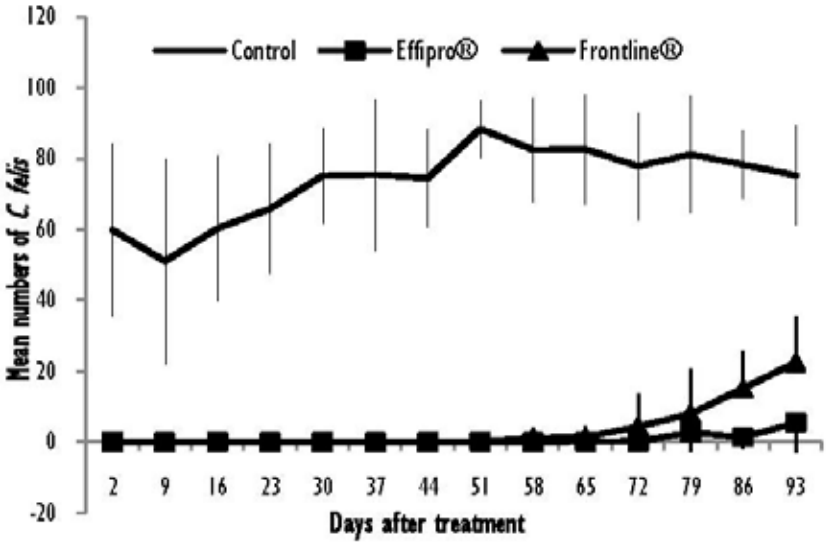

***Table 1**. Means (standard deviation; n=8), median, minimal and maximal values and P-values (using a Kruskal-Wallis test) of weight, average hair length and pre-treatment flea count from dogs treated with one of the two 10% w/v fipronil-based spot-on solutions or left untreated.*

Variable	Treatment group	Mean (std)	Median	Min-Max	P-value
Weight (kg)	Negative control	10.20 (2.39)	9.30	7.80-14.90	0.8331
	Effipro®	10.48 (2.21)	10.45	7.50-14.60	
	Frontline®	11.24 (3.36)	10.30	7.20-17.90	
Average hair length (mm)	Negative control	21.3 (3.4)	21.0	17.5-28.8	0.3326
	Effipro®	21.2 (11.8)	16.3	11.8-44.5	
	Frontline®	17.0 (5.9)	16.0	9.5-27.5	
Pre-treatment flea count	Negative control	69.9 (11.9)	72.5	52.0-86.0	0.3677
	Effipro®	78.0 (9.8)	78.5	58.0-92.0	
	Frontline®	69.8 (22.3)	73.0	27.0-94.0	

***Table 2**. Mean geometric efficacy (%) of two 10% w/v fipronil-based spot-on solutions applied to dogs experimentally infested with* Ctenocephalides felis*, calculated 48 hours after the treatment and 48 hours after each weekly re-infestation over 13 weeks.*

	Days after treatment													
Formulation	2	9	16	23	30	37	44	51	58	65	72	79	86	93
Effipro ®	99.7	100	99.8	100	100	100	99.9	100	100	99.9	99.8	98.9	99.2	97.1
Frontline®	100	100	100	100	100	100	100	100	99.6	99.3	98.2	96.4	85.5	75.4

percent. The application of the two formulations of fipronil led to the complete eradication of all fleas but two (collected from one dog in group 2) when dogs were examined on day 2. The mean therapeutic efficacy was 99.7 percent in group 2 and 100 percent in group 3. The difference between the two groups was not statistically significant (p = 0.35). Dogs in group 2 were protected from re-infestations for 93 days with an efficacy of at least 95 percent. Dogs in group 3 were protected from re-infestations for 79 days with an efficacy of at least 95 percent. Efficacy was further reduced to 88.5 per cent on day 86 and 75.4 percent on day 93. Differences between the two groups were significant on days 86 and 93 (p = 0.0001 and 0.0023, respectively).

DISCUSSION

Both products were well tolerated by all the animals that received them. A total of five dogs were excluded from the study as they developed severe clinical signs consistent with FAD. Four were untreated and one had received Frontline® Top spot. It is understandable that an individual, possibly hypersensitive to fleas, that was treated almost three months before, might develop severe dermatologic signs following a massive infestation (100 unfed fleas). Moreover, this enhances the necessity for dogs with FAD to be treated drastically, either applying a product more frequently than what the manufacturer recommends (every 2-3 weeks), or better combining two different molecules with a different mode of action.[11]

Effipro® Spot-on and Frontline® Top spot were both very effective treatments for flea infestation in dogs (efficacy of 99.7 and 100 percent respectively on day 2). This level of control is comparable to the efficacy of Frontline® Spot-on reported in various similar studies against experimental flea infestations in dogs[12] or in semi-field studies.[13]

Under the conditions of this study, both treatments provided long-lasting residual protection against flea infestations (93 and 79 days for Effipro® Spot-on and Frontline® Top spot, respectively). However, the

standardized procedures, the absence of re-infestations from the environment or from other animals, the climatic stability, and the absence of bathing/swimming or other skin interventions, which could impair the diffusion of the product and/or its persistence on the skin, make the conditions of the present trial ideal. Therefore, under field conditions, such a long-lasting residual protection is unlikely. Hence the information on both products' data sheets which indicate that the insecticidal efficacy against new infestations with adult fleas persists for up to eight weeks. Furthermore, recommendations of monthly applications are commonly made, based on previous field studies.[8,14,15]

Both Effipro® Spot-on and Frontline® Top spot are 10% w/v fipronil-based spot-on solutions but some of their vehicles are different. The present study shows that despite different vehicles, the two formulations were equally able to eradicate flea infestation, and prevent new infestations. They were also equally well-tolerated.

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