Antiparasitic Efficacy of a New Fipronil-based Spot-on Formulation on Dogs Experimentally Infested with Ticks *Dermacentor reticulatus*

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ABSTRACT

In this blinded, randomized, monocentric study, we evaluated the antiparasitic efficacy of a new fipronil formulation (Effipro® spot-on, Virbac) on dogs experimentally infested with ticks, *Dermacentor reticulatus*. The study was performed on three groups (three males and three females per group) of beagles: untreated control, reference drug-treated, and test drug-treated group. The reference drug (Frontline® spot-on, Merial) was administered in the same recommended manner as for the test drug, ie, once on Day 0 at a dose of 1.34 mL/dog.

Dogs were repeatedly infested with ticks (25 males and 25 females) on Days -7, -2, 7, 14, 21, 28, 35, 42, 49, 56, and 63. Except for the Day -2 infestation, ticks were collected 2 days (48 ± 2 hr) after each infestation, and counted according to their viability, attachment, and engorgement status. The ticks applied on Day -2 were removed and counted on Day 2 or 48 ± 2 hr post-treatment. All dogs were monitored for possible adverse events in addition to their general and specific health status.

Our data indicated that compared with the untreated control, both Frontline® and Effipro® group animals showed ~100% reduction of viable tick counts for up to 6 weeks post-treatment ($P \leq 0.005$). Afterwards, both Frontline® and Effipro® showed slightly reduced acaricidal activities, eg, 97.3% and 96.3%, respectively, at the 8-week time point. The immediate as well as sustained antiparasitic activities of the two drugs were consistent and indistinguishable from each other throughout the observation period of 65 days. No adverse reactions associated with the drugs were observed in any of the treated animals.

INTRODUCTION

Ticks are among the most wide-spread ectoparasites, and play an important role as vectors for a number of debilitating diseases in wild, farm, and companion animals.[1-3] Major tick-borne diseases affecting dogs include babesiosis (*Babesia canis*), borreliosis (*Borrelia burgdorferi, B. afzelli*), monocytic ehrlichiosis (*Ehrlichia canis*) and granulocytic anaplasmosis (*Anaplasma phagocytophilum*), not to mention other rickettsial and viral diseases. The majority of the tick-borne diseases are zoonotic, implying that an adequate control of the canine diseases is a necessity not only for the well-being of the animals, but also for the prevention of human infections.[4]

The current range of antiparasitic products in veterinary use relies on various types of molecules that can be used singly or in combination, eg, dympylate, fipronil, imidacloprid, S-methoprene, permethrin, pyriprole, pyriproxyfen, and selamectin. Fipronil, a member of phenylpyrazole family molecules, was first developed into commercial products (Frontline® spray and spot-on, Merial) and they proved to be highly efficacious on dogs against fleas and ticks.[5, 6] Lately, a new fipronil-based spot-on product was developed (Effipro®, Virbac), and the present study was conducted to evaluate its antiparasitic efficacy on dogs against the tick, *Dermacentor reticulatus*, one of the most common ticks in Europe.

MATERIALS AND METHODS

In this blinded, randomized, mono-site study, the antiparasitic efficacy of the test drug was assessed in comparison with two controls: one negative (untreated) and the other positive (reference drug treated). The study was conducted on beagles (nine males aged 8 - 13 months, nine females aged 11 - 12 months) housed in individual pens, under the conditions complying with Ethics Committee recommendations (Charles River Laboratories) and GCP guidelines.[7]

The dogs were randomly allocated into three groups of three males and three females: control (C-group), reference (R-group), and test drug (T-group). On Day 0, dogs received a single treatment with either the test drug or the reference drug. The personnel administering treatments, performing tick counts, or monitoring daily health and clinical status was blinded to the grouping scheme.

The test drug was a spot-on formulation containing fipronil at 10 g/100 mL (Effipro® spot-on, Virbac) as was the reference drug (Frontline® spot-on Dog, Merial). Both drugs (monodose pipettes) were administered directly on the skin between the shoulder blades according to the same recommended posology, 1.34 mL per dog weighing over10 kg and up to 20 kg (0.335 – 0.067 mL/kg body weight).

Dogs were repeatedly infested with unfed adult *D. reticulatus* (25 ± 2 females, 25 ± 2 males) on Days -7, -2, 7, 14, 21, 28, 35, 42, 49, 56, and 63. Briefly, dogs were first sedated by IM administration of ketamine and xylazine. Then the ticks were gently applied to the dorsal or lateral rump area of the dogs in their pen, allowed to crawl into the hair coat and to select their attachment sites. Except for the Day -2 infestation, ticks were collected 2 days (48 ± 2 hr) after each infestation, and categorized according to their viability (alive or killed), attachment status (attached or unattached), and engorgement status (engorged or unengorged). The ticks applied on Day -2 were removed and counted on Day 2 (48 ± 2 hr post-treatment). Tick infestations were taken to be successful when the attachment rates (= attached/applied) were ≥25%. Safety was evaluated via body weight (Day -7, -1, 13, 27, 37, and 69), clinical status (Day 0, 1, and 2), and general health status (daily from Days -7 to 69) as well as specific application site observations (1 hr, 3 hr, 6 hr, 24 hr, and 48 hr post-treatment).

Antiparasitic efficacy (R- and T-group) was defined as the percentage reduction of the number of live attached ticks (engorged or unengorged) and live free ticks in comparison with those of the untreated control (C-group) as follows:

• Efficacy (% reduction) = 100 x [(Mc – Mt)/Mc]

where Mc = mean (geometric) count of C-group, Mt = mean (geometric) count of the test group (R or T).

Statistical analyses were performed with Minitab version 14 (Minitab SARL, France), essentially as described earlier.[8] Briefly, individual as well as all group comparisons were made using a Kruskal-Wallis test or Mann-Whitney U-test (two-tailed). If one group contained a zero tick count, the Kruskal-Wallis test was applied using geometric means (GM) that were calculated by adding 1 to all of the numbers and subtracting it afterwards. For all analyses, the significance threshold was set to $\alpha = 0.05$.

RESULTS

Prior to the drug treatment, the three group animals were statistically indistinguishable ($P > 0.5$) with regard to age, sex, and body weight (Table 1). Clinical status examined on Day 0 (+1 hr, 3 hr and 6 hr), Day 1 (+24 h), and Day 2 (+48h) was normal for all dogs. The examination comprised 1. general behaviour (mood, posture),2. salivation, 3. pupil constriction, and 4. nervous signs.

The rates of tick infestation on Day -5 ranged from 40.3 to 42.0% in a manner statistically indistinguishable among the groups ($P = 0.90$). During the course of the study, tick attachment rates in the control group ranged from 29.7 to 59.6% (mean of 50.5%), satisfying the preset criteria (≥ 25%) for successful challenge infestation.

The body weights of the dogs measured on Day -1 and Day 69 showed that the dogs gained weights of roughly 5.2% (Table 1). There was no significant difference in the weight gain of the animals between three

Table 1: *Baseline status of some general and demographic parameters of the dogs in C (negative control), R (positive control : Frontline®) and T (test : Effipro®) groups. Body weights of the dogs were also measured at the end of the study to check the level of weight gains (Day 69 minus Day -1).*

Parameter		Group (n=6)			P value (all groups)
		C	R	T	
Age [months]	Mean	11.2	12.0	11.5	0.54
	SD	1.72	0.89	0.55	
	min/max	8/13	11/13	11/12	
Sex	Female	3	3	3	*a
	Male	3	3	3	*a
Body weight [kg]	D-1 Mean	11.7	12.1	12.4	0.82
	SD	1.23	1.45	2.57	
	min/max	10.4/13.6	10.6/14.6	10.3/17.1	
	D69 Mean	12.4	12.9	12.8	0.66
	SD	1.31	1.04	1.61	
	min/max	10.8/14.1	11.3/13.8	11.3/15.7	
	Gain Mean	0.7	0.8	0.4	0.82
	SD	1.2	1.2	1.1	
Tick attachment rate [%]	D-5 Mean	42.0	42.0	40.3	0.90
	SD	15.1	9.9	13.0	
	min/max	26/62	26/54	28/64	

**a: No statistics were performed since all groups were equal.*

groups (P = 0.82).

After the single topical administration of drugs, R (Frontline®) and T (Effipro®) groups showed 99.2% and 98.3% reductions, respectively, in viable tick counts on Day 2 and then 100% reductions ($P \leq 0.005$) in both groups up to 6 weeks post-treatment (Figure1). Afterwards (Days 44, 51, 58, and 65), the acaricidal efficacies (%) diminished slightly: R-group (99.1, 96.2, 97.3, and 89.8, respectively) and T-group (97.4, 98.8, 96.3, and 85.6, respectively).

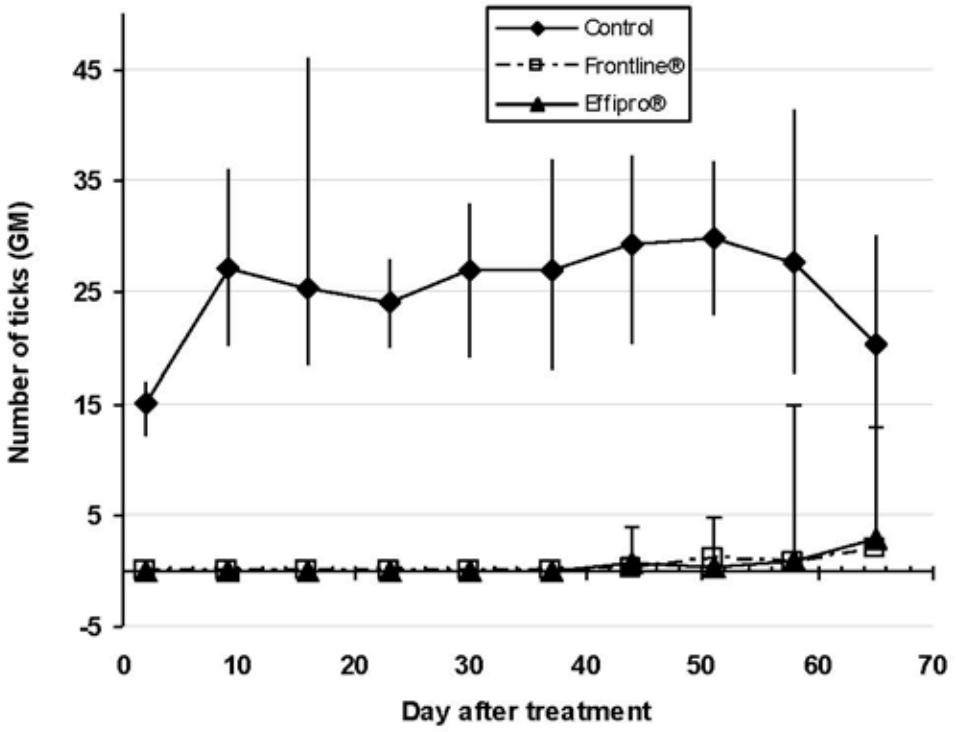
The antiparasitic efficacies of the two drugs were indistinguishable throughout the study period (P values ranging from 0.2 to 1).

During tick recoveries, one or two dead engorged ticks were found attached to the dogs sporadically (on Days 2 and 6 in C-group, on Days 16 and 65 in R-group, and on Day 23 in T-group), but they were statistically insignificant. No adverse reactions nor application site abnormalities (eg, alopecia, erythema, and oedema) associated with the drug treatments were observed in any of the groups throughout the study.

DISCUSSION

The test drug (Effipro® spot-on) is a newly developed product containing fipronil as the active agent, as does the reference product (Frontline® spot-on Dog). In this comparative study, the initial conditions of the dogs with respect to physical and demographic parameters were strictly comparable among the three groups. Against these backgrounds, our data showed unambiguously that 1. both drugs were immediately active and virtually 100% protective against *D. reticulatus*

Figure 1: *Antiparasitic efficacies on D. reticulatus of R (Frontline®, -□-) and T (Effipro®,-▲-) against C (untreated control, -♦-) group. Both live attached ticks (engorged or unengorged) and live free ticks were collected 48 hr after each tick infestation (except for the first one on Day -2) and the number of ticks (GM for geometric mean) was plotted against the study day. Error bars indicate the range (min/max) of the number of recovered ticks at each time point.*



for up to 6 weeks post-treatment, 2. they remained highly protective (>96%) at the 8-week time point, 3. antiparasitic efficacies of the two drugs were indistinguishable from each other throughout the study period, and 4. no drug-related adverse events were observed.

The number of ticks collected on Day 2 (C-group) was ~40% lower than those (C-group) of the other time points. Considering that the Day 2-counts were obtained 4 days after the infestation as compared to 2 days for all other time points, the low counts are attributed to non-drug related reduction of ticks occurring within the first 2 days of infestation.

CONCLUSION

Our present results corroborate and support the observations made in earlier studies on antiparasitic efficacies of Effipro® spot-on. For instance, in a study closely resembling the present one in experimental design but with another tick species Ixodes ricinus, both drugs (Effipro® spot-on and Frontline® spot-on) were shown to be protective up to 5 weeks with >95% efficacies.8 In another comparative study on dogs evaluating the efficacy against flea *Ctenocephalides felis*, both drugs showed immediate as well as sustained protective activities of >95% for 80 days.[9] The available data thus support the notion that Effipro® spot-on was equally safe and efficacious on dogs as Frontline® spot-on against major ectoparasites such as ticks (*D. reticulatus* and *I. ricinus*) and fleas (*C. felis*).

It should be noted, however, that both drugs (Frontline® and Effipro®) did not prevent the ticks from attaching to the animals. Nevertheless, those ticks that attached to the drug-treated animals could be killed within the first 24 to 48 hr. It means that ticks are killed usually prior to engorgement, thereby minimizing but not excluding the risk of disease transmission.

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