

Long-term Efficacy Against Fleas (*Ctenocephalides felis*, Bouché 1835) of Monthly Topical Treatments with Fipronil Based Spot on Formulations Compared to a Flumethrin/Imidacloprid Impregnated Collar on Dogs Subjected to Regular Water Exposure

Lénaïg Halos^{a*}

Josephus Fourie^b

Ina Bester^b

Matthias, Pollmeier^a

Frédéric Beugnet^a

^aMerial S.A.S., 29 Av Tony Garnier, 69007, Lyon, France.
Lenaig.halos@merial.com

^bClinVet International (Pty) Ltd, Uitsig Road, Bainsvlei,
9321 Bloemfontein, Republic of South Africa.

* Corresponding author

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ABSTRACT

The objective of this study was to compare the long term efficacy against fleas (*Ctenocephalides felis*) of repeated monthly topical treatments with Fipronil based spot-on products (Certifect[®] followed by Frontline[®] Combo, both Merial) to the efficacy of a flumethrin/imidacloprid impregnated collar (Seresto[®], Bayer Animal Health) on dogs exposed to simulated rain. The two fipronil based spot-on products contain the same

amounts of fipronil and (S)-methoprene, and are registered in Europe with a one-month flea control claim while the flumethrin/imidacloprid impregnated collar has a flea efficacy claim of 7 to 8 months. Eighteen dogs were followed during an 8 months period. Six dogs remained untreated, 6 dogs were fitted with the imidacloprid/flumethrin collar on Day 0, and 6 dogs were treated monthly for 3 months with fipronil-based formulations starting on Day 120 (Certifect[®] on Days 120 and 150 followed by Frontline[®] Combo on Days 181 and 210). Flea infestations comprising 100 *C. felis* each were performed twice a month starting on Day 134 and repeated through Day 231. Dogs were showered with water for 1 minute at fort-

nightly intervals starting the week after each treatment throughout the study. The efficacy of monthly administered fipronil-based spot on products was not impacted by the simulated rain as confirmed by reductions in *C. felis* counts between 96% to 100% at all timepoints. The efficacy of the flumethrin/imidacloprid impregnated collar was markedly impacted by the simulated rain with reductions in *C. felis* counts of between 31.7% to 64.8% versus control following challenge in months 4-8. The efficacy of the fipronil-based spot-on products was significantly higher ($p < 0.05$) than the efficacy observed with the flumethrin/imidacloprid impregnated collar at all challenge times.

INTRODUCTION

Fleas, particularly those of the genus *Ctenocephalides*, are the most common ectoparasites of dogs and cats worldwide. Flea infestation can cause considerable irritation to animals and humans, or can lead to severe disorders such as anemia and dermatological problems including flea allergic dermatitis (FAD). It is estimated that flea related diseases are responsible for almost 50% of dermatological cases reported to veterinarians (Rust and Dryden, 1997; Guaguère and Beugnet, 2006).

Many flea species are of veterinary and public health importance as they can be reservoirs and potential vectors for a variety of pathogens including zoonotic agents. The cat flea, *Ctenocephalides felis*, is a known vector of *Bartonella henselae*, *B. clarridgeiae*, and *Rickettsia felis*, which in humans can cause cat scratch disease, endocarditis, and flea borne spotted fever, respectively. The fleas of dogs and cats are also known as intermediate hosts of *Dipylidium caninum* (Beugnet and Fourie, 2013; Dryden and Hodgkins, 2010).

Because of animal and human welfare, controlling fleas is of great importance worldwide. Despite the increasing number of flea control products and their use, flea infestation of dogs remains common in Europe and other continents (Rust, 2005).

Several insecticides, alone or in combi-

nation products, which are appropriate and safe to treat domestic dogs, have been formulated for application either topically (spot on, collar) or more recently orally (Rust, 2005; Horak *et al*, 2012; Beugnet and Franc, 2012). The insecticide impregnated collars claim to be effective for several months.

Although its long lasting efficacy has been tested and validated before registration, the duration of efficacy may be challenged under conditions of normal use, including exposure to factors like rain, water immersion, UV degradation, etc. Spot on formulations have a shorter duration of action, ie, generally 1 month, and it could be hypothesized that the efficacy would remain more stable in the face of environmental factors.

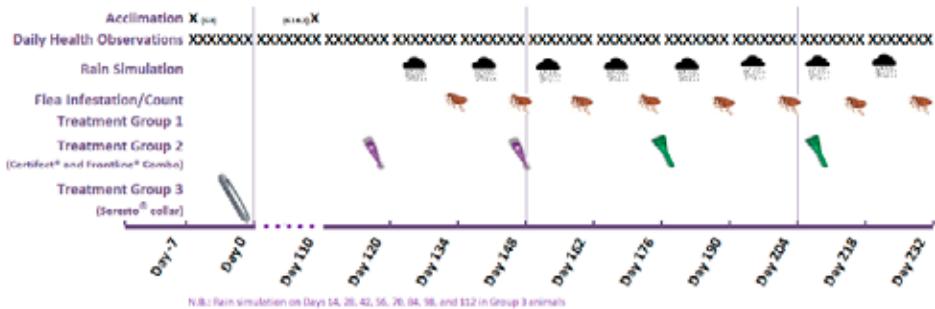
The aim of the present investigation was to evaluate the long-term flea efficacy of a flumethrin/imidacloprid impregnated collar (Seresto[®], Bayer Animal Health) that is applied once compared to the efficacy of fipronil-based spot on formulations (Certifect[®] and Frontline[®] Combo, Merial) applied monthly to dogs under experimental conditions mimicking natural water exposure (twice a month exposure to simulated rain). Certifect[®] and Frontline[®] Combo have been demonstrated to provide similar efficacy against fleas as the formulation containing fipronil as well as the dose delivered to dogs is the same for both products. The two spot-on products are registered in Europe with a 1-month flea control claim while the impregnated collar has a 7 to 8-months flea efficacy claim. Based on Seresto[®] s' claim that flea efficacy gradually decreased following monthly shampooing or water immersion starting in the 5th months, it was assumed that

- the collar would be fully efficacious for at least 3 months and that
- the efficacy of the spot-on products would be comparable each month, challenge flea infestations were therefore initiated from the fourth month.

MATERIALS AND METHODS

The study was designed in accordance with the "World Association for the Advancement

Figure 1: Study Design



N.B.: Rain simulation on Days 14, 28, 42, 56, 70, 84, 98, and 112 in Group 3 animals

of Veterinary Parasitology (W.A.A.V.P) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats” (Marchiondo *et al*, 2013), and was conducted in accordance with Good Clinical Practices as described in the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guideline GL9 (EMA, 2000). All dogs were managed similarly, with due regard for their well-being and in compliance with Merial and local Ethics Committee approvals.

Dogs

Eighteen healthy mixed breed dogs (12 males/6 females) aged at least 3 months old were enrolled in the study. Dogs treated with ectoparasiticides (either topical or systemic) within 3 months before the start of the study were excluded. Seresto® collar treated dogs had a physical examination on Day -7 to ensure a healthy status. Untreated and fipronil treated dogs had a physical examination on Day 113. None of the dogs were debilitated, suffering from disease or injury, or otherwise unsuitable for inclusion in the study. None of the dogs included presented abnormalities in the neck area where products were to be applied.

Treatment Administration

Six dogs formed the untreated control Group 1. Six dogs in Treatment Group 2 (fipronil-based spot on products) were treated with the appropriate commercially available pipette size of Certifect® on Day 120 and Day

150 and Frontline® Combo on Day 181 and Day 210, according to the registered label instructions. Six dogs in Treatment Group 3 (flumethrin/imidacloprid impregnated collar) were fitted with the commercially available Seresto® collar following the label instructions on Day 0.

Following treatments, all dogs were observed hourly (±30 minutes) for 4 hours after the last animal was treated.

Experimental Study Design

The impregnated collar treated Group was allocated on Day -5, the control and fipronil-based spot-on product treated Groups were added to the study on Day 115 (and acclimated from Day 110) (see Figure 1 for study design).

Flea Infestations and Counts

Adult *C. felis* were used for artificially induced infestations. These fleas were from a South African strain that is not known to be resistant to any ectoparasiticide. All dogs were infested every 2 weeks with 100 adult *C. felis* fleas starting on Day 134 (Days : 134, 148, 161, 175, 189, 203, 217, and 231), followed by comb counts 24 (±2) hours post infestation (Figure 1). Each dog was combed for a minimum of 10 minutes using a fine flea comb. If fleas were found during these 10 minutes, then the dog was combed for 15 minutes. However, if no fleas were found on the dog in these 10 minutes, then the count was stopped. The dog’s entire coat was combed thoroughly. To prevent cross-contamination between treatment groups, separate flea combs and lab coats were

Table 1: Summary of mean flea counts and percent efficacies against fleas on dogs treated with Fipronil 10% w/v combination products or flumethrin/imidacloprid collar.

DAY	GROUP 1 Negative control n =6	Dogs Treated with Certifect® on Day 120 and Day 150 and Frontline® Combo on Day 181 and Day 210n =6			Dogs Treated with Seresto® at Day 0 n =6	
		Flea counts (arithmetic mean)	Flea counts (arithmetic mean)	Efficacy (%) Arithmetic mean (Geo mean)	Flea counts (arithmetic mean)	Efficacy (%) Arithmetic mean (Geo mean)
135	74.0	0.0	100.0 (100)	42.3	42.8 (45.1)	
149	66.7	4.2	93.8 (96.8)	47.2	29.3 (31.7)	
162	60.2	0.0	100.0 (100)	33.8	43.8 (48.0)	
176	67.2	2.2	96.8 (98.3)	40.2	40.2 (47.9)	
190	62.3	0.0	100.0 (100)	39.2	37.2 (53.9)	
204	39.8	0.8	97.9 (98.8)	20.7	48.1 (64.8)	
218	59.3	0.0	100.0 (100)	39.0	34.3 (40.3)	
232	67.7	3.3	95.1 (97.5)	44.2	34.7 (34.1)	

used for each treatment group. Gloves were changed systematically between each dog.

Rain Simulation

Water showering was carried out in a designated area by wetting dogs, including the head, with a gentle spray from a bathing wand for one minute. The dogs were then dried smoothly using a blow-dryer before returning them to their cages. Dogs fitted with the collar were showered every two weeks starting on Day 14 (Days 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 155, 170, 184, 198, 212, and 226). Dogs treated with Fipronil were showered at 14 days intervals starting D126 through day 226 (Days 126, 140, 155, 170, 184, 198, 212, and 226) (Figure 1).

Data Analysis

The flea counts were transformed to the natural logarithm of (count +1) for calculation of geometric means by treatment group at each time point. Arithmetic means were also calculated. Percentage reduction from the untreated control group (Treatment Group 1) mean was calculated for Treatment Groups 2 and 3 at each counting time point using

the formula $[(C - T) / C] \times 100$, where C was the geometric or arithmetic mean for the untreated control group and T is the geometric or arithmetic mean for Treatment Groups 2 or 3. Each treated Group was compared to the untreated control group as well as other treated group using Analysis of Variance on log count (ANOVA). All testing was two-sided at the significance level $\alpha=0.05$.

Results

The adult flea counts at 24 hours post infestation and efficacy results throughout the study are summarized in Table 1. The arithmetic mean flea counts of untreated dogs ranged from 39.8 to 74. The fipronil-based spot on product treated dogs had statistically significantly ($p<0.05$) lower flea counts (based on geometric means) compared to the negative control Group on all assessments days. The flea counts were not significantly different between the four spot on treatment time points supporting the fact that the two fipronil-based products provide similar efficacy. The imidacloprid/flumethrin treated dogs had statistically significantly ($p<0.05$)

less fleas (based on geometric means) compared to the negative control Group on Days 135, 162, 204 and 232 but not at the other time points. The fipronil-based spot on product treated dogs had statistically significantly ($p < 0.05$) less fleas (based on geometric means) compared to the imidacloprid/flumethrin treated dogs on all assessment days throughout the study.

The fipronil-based spot on product treatment provided 96 to 100% efficacy against fleas (based on geometric means), whereas the imidacloprid/flumethrin impregnated collar provided 31.7% to 64.8% efficacy against challenge starting on Day 134. No adverse events or other health problems were observed throughout the study.

DISCUSSION

This study demonstrated the efficacy of monthly applications of fipronil-based combination products, i.e., Certifect® and Frontline® Combo, in the control of flea infestations in dogs subjected to simulated rain. Four successive monthly treatments either with Certifect® or Frontline® Combo offered a similar sustained flea efficacy of >96% over a period of 4 months. Following a monthly schedule of application, these spot on products were significantly more effective against *C. felis* on dogs exposed to simulated rainfall twice a month than the flumethrin/imidacloprid impregnated collar exposed to simulated rain twice a month when efficacy was evaluated between 19 and 33 weeks after application.

Collars are often considered as inexpensive devices for the prevention of ectoparasite infestations, including fleas, due to their long lasting activity, i.e. several month of prophylactic efficacy. This long duration is due to the slow release of the actives from the matrix of the collar. Nevertheless, under natural conditions of use, many factors, such as insufficient skin contact, wear by friction, regular water immersions, or mechanical deteriorations may impair the sustained efficacy and lead to variable protection over time.

In previous studies, flumethrin/imida-

cloprid impregnated collars demonstrated 93.2% efficacy on Day 177 (6 months) and 94.5% on Day 191 against fleas under experimental conditions without water exposure (Horak *et al*, 2012). In the present study, dogs were exposed to simulated rain for one minute representing a non-severe water exposure scenario. Nonetheless, the efficacy decreased dramatically to level below 50% (arithmetic mean) after 4 months with exposure to simulated rain. In contrast, topical spot on products usually have a duration of prophylactic efficacy that extends to 1 month or more. Fipronil translocates on the skin within 24 hours and accumulates in skin lipids. Its lipophilic properties and dose were studied to provide a month of protection against fleas and ticks under natural conditions including water exposure and shampooing (Beugnet and Franc, 2012). Water immersion is an important factor to be considered when assessing the efficacy of an ectoparasiticide in the field. Indeed, animals exposed to parasitic pressure usually have an outdoor lifestyle and are therefore often subjected to natural conditions including rain, swimming and bathing. This study underlines the need to choose the appropriate antiparasitic treatment adapted to each dog's lifestyle and environment.

CONFLICT OF INTEREST

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