Scientific Research Publishing

Open Journal of Veterinary Medicine, 2018, 8, 65-74
http://www.scirp.org/journal/ojvm
ISSN Online: 2165-3364
ISSN Print: 2165-3356

Comparative Efficacy of Frontline Tri-Act® Spot on (Fipronil/Permethrin) versus Seresto® Collar (Imidacloprid/Flumethrin) against Fleas (*Ctenocephalides felis*) and Ticks (*Rhipicephalus sanguineus*) on Dogs with Simulation of Bi-Monthly Rain Exposure

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How to cite this paper: Beugnet, F., Servonnet, M., Halos, L. and Lebon, W. (2018) Comparative Efficacy of Frontline Tri-Act® Spot on (Fipronil/Permethrin) versus Seresto® Collar (Imidacloprid/Flumethrin) against Fleas (*Ctenocephalides felis*) and Ticks (*Rhipicephalus sanguineus*) on Dogs with Simulation of Bi-Monthly Rain Exposure. *Open Journal of Veterinary Medicine*, **8**, 65-74.
https://doi.org/10.4236/ojvm.2018.85008

Received: April 24, 2018
Accepted: May 27, 2018
Published: May 30, 2018

Abstract

The objective of the study was to compare the efficacy of one topical insecticide-acaricide (Frontline Tri-Act®) and of one collar (Seresto®) against fleas (*Ctenocephalides felis*) and ticks (*Rhipicephalus sanguineus*) on dogs exposed to bi-weekly water showering. Twenty four (24) dogs were enrolled in the study. A first set of 16 dogs were acclimatised to their cages from Day 1 to 7 and a second set of 8 dogs from Day 163 to Day 169. The 24 dogs were randomly allocated to three groups (1 to 3). Dogs assigned to Group 1 were not treated and served as negative controls. Dogs assigned to Group 2 received the Seresto® collar on Day 0 and dogs in Group 3 received Frontline Tri-Act® on Days 170 and 198. The dogs were observed hourly for four hours after treatment administration for possible adverse reactions. Dogs in Groups 1 and 2 underwent water showering on Days 14, 28, 42, 56, 70, 84, 98, 112, 126, 143 and 157. Dogs in all groups underwent water showering on Days 173, 185, 199 and 213. Dogs were infested with approximately 100 (±4) adult, unfed *C. felis* fleas only on Days 177, 190, 203, and 217, in alternance with infestations with 50 adult unfed *R. sanguineus* on Days 182, 196, 210, and 224, to assess sustained efficacy. Fleas and ticks were removed and counted on 24 and 48 hours ± 2 hours after each infestation, respectively. Frontline Tri-Act® was >99% effective against *C. felis* following an initial and a second monthly administration on dogs that were water showered bi-weekly. The Seresto® collar was from 68.3% to 92.9% effective against *C. felis* Days 178 to 218 after collar administration. Frontline Tri-Act® was effective from 87.8% to 100% against

ticks whereas Seresto collar was effective from 82.2% to 94.2% from Day 184 to Day 226.

Keywords

Efficacy, Dogs, Seresto®, Frontline Tri-Act®, *Ctenocephalides felis*, *Rhipicephalus sanguineus*

1. Introduction

Fleas of the genus *Ctenocephalides* are the most common ectoparasites of dogs and cats worldwide [1]. 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) [1]. 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 [1] [2] [3]. 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* [2].

Ticks are among the second most common external parasites of dogs. They have the potential to transmit pathogenic agents to both dogs and their owners. *Rhipicephalus sanguineus* has a world-wide distribution. It can transmit a variety of pathogens to dogs, including *Babesia vogeli*, *Ehrlichia canis*, *Anaplasma platys*, and *Hepatozoon canis*. It is also capable of transmitting pathogens to humans such as *Rickettsia conorii*, the agent of Mediterranean spotted fever [4] [5] [6].

In that context, the role of an anti-flea and tick product is not only to treat an existing infestation but more importantly, to protect the animal from re-infestation and potential concomitant vector-borne disease transmission or development of allergy [1] [2]. Despite the increasing number of ectoparasiticide products and their use, flea and ticks infestation of dogs remains common in Europe and other continents [7]. Many ectoparasiticides have been formulated for topical application (spot ons, collars) or more recently for oral administration [8] [9]. The topical products, spot ons and collars, that act by direct contact with ectoparasites might have a better speed of kill due to their immediate efficacy compared to oral systemic product that requires that the parasite start to take a blood meal prior to being affected [1] [7] [8] [9]. The insecticide-acaricide impregnated collars claim to be effective for several months. Although its long lasting efficacy has been tested and validated before registration, the release of active ingredients by collars and their duration of activity may be impacted by external factors like mechanical attrition, water immersions, and rain [10] [11]. Conversely, monthly re-applications of a topical spot on may reduce the impact of such external factors. This has been previously demonstrated by comparing

the flumethrin/imidacloprid collar to two fipronil based products (Frontline Combo® and Certifect®) [11]. Recently, a spot on formulation combining fipronil and permethrin (Frontline Tri-Act®) has been developed for use as a monthly topical solution for the control of fleas, ticks, mosquitoes, sandflies and biting flies in dogs [12]-[18].

The aim of the present investigation was to evaluate the long-term flea and tick efficacy of a flumethrin/imidacloprid impregnated collar (Seresto®, Bayer Animal Health) that is applied once compared to the efficacy of the fipronil-permethrin spot on formulation (Frontline Tri-Act®, Boehringer-Ingelheim Animal Health) applied monthly to dogs under experimental conditions mimicking natural water exposure (dogs exposed to bi-monthly water showering).

2. Materials and Methods

The study was designed in accordance with the "World Association for the Advancement 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" [19], 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 (EMEA, 2000) [20] [21]. All dogs were managed similarly, with due regard for their well-being and in compliance with Merial and local Ethics Committee approvals. All dogs were identified by a microchip and an identification (ID) number.

This study was a non-blinded, randomised and negative controlled efficacy study. The study was conducted in three groups of eight dogs each (mongrels and Beagles, males and females), weighing 10 to 27.7 kg. Dogs were included in the study if they had been acclimatised to the study site for at least seven days; they were clinically healthy as verified by a Veterinarian on Day 7 or Day 163; they were ≥6 months old; they weighed ≥ 8 kg on Day 4 or 168; females were not clinically pregnant; they had not been treated with a long acting topical or systemic acaricide/insecticide during the 12 weeks preceding administration day (Day 0 or Day 170).

During the acclimatisation period for Groups 1, 2, and 3, an initial *C. felis* flea infestation was performed to evaluate the susceptibility of each dog to experimental infestation and for random allocation to the groups.

Dogs assigned to Group 1 were not treated and served as negative controls. Dogs assigned to Group 2 received the Seresto® collar (imidacloprid—flumethrin) on Day 0, and dogs in Group 3 received Frontline Tri-Act® (fipronil—permethrin) on Day 170 and 198. The dose administered was calculated according to the dog's individual body weight following the European labelling (**Table 1**). The dogs were observed hourly for four hours after treatment for possible adverse reactions.

Table 1. Product dose rate.

Group	Day	Sample size	IVP	Active ingredient (s)	Dose rate
2	0	8	Seresto®	4.5% flumethrin and 10.0% imidacloprid (w/w)	One collar per dog weighing ≥ 8 kg
3	170 and 198	8	Frontline Tri-Act®	6.76% fipronil and 50.48% permethrin (w/v)	2 mL for dogs weighing 10 kg to 20 kg 4 mL for dogs weighing > 20 kg to 40 kg

Dogs were infested with 100 (±4) adult unfed *C. felis* fleas only on Day 7 (dogs enrolled were to be included in Groups 1 and 2) or Day 163 (dogs enrolled were to be included in Groups 3 and 4), for randomisation purposes, and on Days 177, 190, 203 and 217, to assess sustained efficacy. Dogs were also infested by 50 adult unfed *R. sanguineus* ticks (sex ratio 50:50) on Days 182, 196, 210, and 224. Fleas were removed and counted on Day 6 or Day 164 (24 hours ± 1 hours after infestation) and on Days 178, 191, 204 and 218 (24 hours ± 2 hours after infestation). Ticks were removed and counted 48 hours after each infestation (*i.e.* Days 184, 198, 212, and 226).

Dogs in Groups 1 and 2 underwent water showering on Days 14, 28, 42, 56, 70, 84, 98, 112, 126, 143 and 157. Dogs in all groups underwent water showering on Days 173, 185, 199 and 213.

Dog with the ID "DF7 98F" (Group 2) managed to remove and destroy its collar and was therefore removed from the study (Day 144) before the end of the study as we could not test anymore insecticidal/acaricidal protection status.

The dog cages were part of an indoor animal unit, environmentally controlled for temperature (20˚C ± 4˚C). A photoperiod of 12 hours light and 12 hours darkness was maintained. The animals were kept individually in cages and no physical contact between dogs was possible. However, animals still had visual and auditory contact with other dogs.

The animals were fed once or twice a day (depending on the age of the dogs) according to the food manufacturer's recommendation.

2.1. Water Showering

Water showering was carried out in a designated area by thoroughly wetting dogs, including the head, with spray from a bathing wand for at least one minute. The dogs were dried with a blow-dryer before being returned to their cages.

2.2. Efficacy Criteria

The assessment criterion was the number of live ticks and fleas counted in the control and the treated group(s) on the various assessment days.

2.3. Flea and Tick Infestations

Laboratory bred strains (European strains) of *C. felis* and *R. sanguineus* were used for all infestations. Each dog was infested with approximately 100 (±4) fleas

or 50 ticks (25 males and 25 females) on the days as set out in Table 2.

Fleas were removed and counted by combing technique 24 ± 2 hours after infestation. The method of combing was by several strokes of the comb in each body area of the animal, each time moving in the same direction, following the pattern of the hair coat. Movement, from one part of the animal'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 once more so that all areas were combed a minimum of two times. If fleas were still present, the combing procedure was continued for a third time or more until no live fleas were found. Ticks were removed by thumbing technique of the complete body of each dog. Ticks were categorized as live or dead and attached or free.

2.4. Statistical Analysis

Body weights measured during the acclimatisation period, were compared between the groups in order to evaluate their homogeneity at the time of inclusion.

With consideration of available guidelines, EMEA/CVMP/005/2000-Rev.3 [20], it was decided that the primary efficacy calculations would be based on arithmetic mean values rather than geometric mean values.

Efficacy against fleas was calculated according to the following formula:

Efficacy (%) against fleas = $100 \times (M_c - M_t)/M_c$, where:

M_c = Mean number of live fleas on dogs in the negative control group (Group 1) at a specific time point.

M_t = Mean number of live fleas on dogs in the IVP groups (Group 2 and 3) at a specific.

Efficacy against ticks was calculated as follows:

Efficacy (%) against ticks = $100 \times (M_c - M_t)/\mathrm{M_c}$, where:

M_c = Mean number of live ticks on dogs in the negative control group (Group 1) at a specific time point.

M_t = Mean number of live ticks on dogs in the IVP groups (Group 2 and 3) at a specific time point.

The groups were compared using ANOVA Test. Groups were homogeneous with regard to flea counts measured during the acclimatisation period. The level of significance of the formal tests was set at 5%, all tests were two sided.

3. Results

No adverse reactions that could be related to any of the treatment were observed during the 8 month study period. Arithmetic mean values of live *C. felis* flea counts, efficacies, and p-values, are summarised in Table 2 and Table 3. Arithmetic mean values of live *R. sanguineus* tickcounts, efficacies, and p-values, are summarised in Table 4 and Table 5. The arithmetic mean values of live flea counts for the negative control group ranged from 70.4 to 81.0, indicating a vigorous flea challenges on all assessment days. Adequacy of infestation (at least

Table 2. Efficacy against fleas based on arithmetic means.

	Control Group 1	Group 2		Group 3	
Day	Mean +/− SD	Mean +/− SD	Percentage efficacy	Mean +/− SD	Percentage efficacy
Day 178	70.4 +/− 11	22.3 +/− 13.2	68.3	0.0 +/− 0	100
Day 191	81.0 +/− 10.8	5.7 +/− 4.3	92.9	0.8 +/− 2.7	99.1
Day 204	79.9 +/− 10.1	8.7 +/− 4.6	89.1	0.0 +/− 0	100
Day 218	76.0 +/− 8.6	15.4 +/− 10.4	79.7	0.0 +/− 0	100

Group 1: Negative control; Group 2: Dogs were treated with Seresto® collar; Group 3: Dogs were treated topically with Frontline Tri-Act®; SD = Standard Deviation.

Table 3. ANOVA test comparison of arithmetic means for fleas.

	ANOVA p-value			
Comparison	Day 178	Day 191	Day 204	Day 218
Group 1 with Group 2	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Group 1 with Group 3	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Group 2 with Group 3	**0.00055**	**0.022**	**0.0021**	**0.00055**

p-value: Wilcoxon rank sum test with continuity correction; Alternative :two. Sided; Group 1: Negative control; Group 2: Dogs treated with Seresto® collar; Group 3: Dogs treated topically with Frontline Tri-Act®; SD = Standard Deviation.

Table 4. Efficacy against ticks based on arithmetic means.

	Control Group 1	Group 2		Group 3	
Day	Mean +/− SD	Mean +/− SD	Percentage efficacy	Mean +/− SD	Percentage efficacy
Day 172	34.1 +/− 5.6	3.6 +/− 3.1	89.5	7.0 +/− 5.8	79.5
Day 184	26.1 +/− 7.2	2.4 +/− 2.1	90.7	0.5 +/− 0.9	98.1
Day 198	33.8 +/− 7.9	6.0 +/− 5.1	82.2	4.1 +/− 4	87.8
Day 212	33.3 +/− 6.5	3.4 +/− 1	89.7	0.0 +/− 0	100
Day 226	32.3 +/− 8.5	1.9 +/− 1.9	94.2	1.4 +/− 2.6	95.7

Group 1: Negative control; Group 2: Dogs treated with Seresto® collar; Group 3: Dogs treated topically with Frontline Tri-Act®.

Table 5. ANOVA test comparison of arithmetic means for ticks.

	ANOVA p-value				
Comparison	Day 172	Day 184	Day 198	Day 212	Day 226
Group 1 with Group 2	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Group 1 with Group 3	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Group 2 with Group 3	0.3493	0.3923	0.5135	0.0595	0.8397

p-value: One-way ANOVA with a treatment effect; Group 1: Negative control; Group 2: Dogs treated with Seresto®collar; Group 3: Dogs treated topically with Frontline Tri-Act®.

50% of the challenge) was demonstrated for all dogs in the negative control group at each assessment time point. The infestation rate for ticks ranged from 26 to 30 in control dogs, also confirming an adequate tick challenge.

Based on arithmetic mean values of flea and tick counts, significantly fewer fleas and ticks were recorded for the treated groups compared to the negative control group throughout the study ($p < 0.05$).

Statistically significantly fewer fleas were present on the dogs treated with Frontline Tri-Act® than the dogs treated with the Seresto® collar ($p < 0.05$) on all time-points. There was not significant difference between the two treated groups in regard to the tick counts at any time-points.

Based on arithmetic mean values of live flea counts, the Seresto® collar was 68.3% to 92.9% effective against *C. felis* from 178 to 218 days after collar administration.

Based on arithmetic mean values of live fleas, Frontline Tri-Act® was 99.1% to 100% effective against *C. felis* after two monthly administrations, despite fortnightly water showering. Frontline Tri-Act® was persistently ≥95% effective against *C. felis* following monthly administration based on arithmetic mean values for live fleas. Based on arithmetic means, Frontline Tri-Act® efficacy ranged from 87.8% to 100% against ticks whereas Seresto collar had efficacy ranging from 82.2% to 94.2% from Day 184 to Day 226.

4. Discussion

This study demonstrated the efficacy of monthly applications of the fipronil-permethrin combination product in the control of both flea and tick infestations in dogs subjected to simulated rain or water immersion. Two successive monthly treatments provided a sustained flea efficacy of >99%, and a tick efficacy > 87.8%. Following a monthly schedule of application, the spot on product was significantly more effective against *C. felis* on dogs exposed to simulated rainfall twice a month than the flumethrin/imidacloprid impregnated collar in its 7th and 8th month, including water showering twice every month during 8 months. No significant differences were observed in regard to tick efficacy.

Collars are often considered as inexpensive devices for the prevention of ectoparasite infestations due to their long lasting activity, *i.e.*, several months of sustained activity. This persistant activity is due to the slow release of the actives from the matrix of the collar [1] [5]. Nevertheless, under natural conditions of use, many factors, such as insufficient skin contact, regular water immersions, or mechanical deteriorations may impair the sustained efficacy and lead to variable protection over time.

In previous studies, flumethrin/imidacloprid 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 [10]. In the previous comparison with Frontline Combo® and Certifect®, including fortnightly water showering like the present study, the collar efficacy ranged from 34.3 to

48% in months 7 and 8 [11]. In the present study, dogs were exposed to water showering bi-weekly for one minute representing a non-severe water exposure scenario. Nonetheless, the collar efficacy against fleas was 79.7% on Day 218 (month 7). The insecticidal efficacy of the collar does not seem to decrease regularly, the efficacy against fleas being better on Days 194 and 204 than on Days 178 and 218. A similar variation in tick efficacy was observed. It may be related to the time of the last water showering and the time for replenishment of the active ingredients from the collar matrix to the skin. Fipronil and permethrin translocate from the topical spot on the skin within 24 hours and accumulate in skin lipids. The fipronil-permethrin formulation was developed to provide a month of protection against fleas and ticks under natural conditions including water exposure and shampooing [16] [17]. Except on Day 198, corresponding to the Day 28 of the first spot on treatment, where the efficacy was slightly below 90%, the efficacy against ticks stayed above 95% at the other time points, corresponding to the Day 14 of the first and second monthly treatment, and the Day 28 of the second treatment. The flumethrin-imidacloprid collar presented two time-points with efficacies below 90%.

5. Concluding Remarks

It can be concluded from this study that 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 [1] [5] [6]. The duration of protection may vary due to the activities of each dog. Monthly topicals may be impacted, but probably less than very long acting collars due to the regular re-applications.

Conflict of Interest

The work reported herein was funded by Boehringer Ingelheim Animal Health. All authors are current employees or contractors.

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