

Comparative Curative Efficacy of Two Spot On Formulations, Fipronil/Amitraz/ (S)-Methoprene and Imidacloprid/ Permethrin, on Two Tick Species in Dogs

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ABSTRACT

This study compared the acaricidal efficacy of a new spot-on formulation of fipronil + amitraz + (S) methoprene (CERTIFECT™) and imidacloprid + permethrin (K9 ADVANTIX®) against a mixed infestation of *Rhipicephalus sanguineus*, the brown dog tick, and *Dermacentor variabilis*, the American dog tick. Twenty-four mixed-breed dogs (15 males and 9 females) weighing 10.7 to 24.9 kg and ranging from 1.4 to 13.8 years of age were divided into three groups of eight dogs. Group 1 was the untreated control group. On Day 0 dogs in Group 2 were

treated with CERTIFECT and Group 3 dogs were treated with K9 ADVANTIX. All dogs were infested pre-treatment with 50 unfed *R. sanguineus* (Day -2) and 50 unfed *D. variabilis* (Day -1). The ticks were thumb counted 24 hours post-treatment and removed and counted 48 hrs post-treatment. The curative efficacy of CERTIFECT against *R. sanguineus* was 82.4% at 24 hrs and 99.1% at 48 hrs, while K9 ADVANTIX curative efficacy was 44.6% at 24 hrs and 75.8% at 48 hrs. Similarly, the percent reduction of *D. variabilis* was 86.0% at 24 hrs and 100.0% at 48 hrs for CERTIFECT and 50.0% at 24 hrs and 75.0% at 48 hrs for K9 ADVANTIX®. CERTIFECT was significantly ($P < 0.05$) more efficacious in reducing the number of *R. sanguineus* and *D. variabilis* both at 24 and 48 hours post-treatment when compared to K9 ADVANTIX.

INTRODUCTION

Ticks and fleas are the most frequent ecto-

®K9 ADVANTIX is a trademark of Bayer.

™Merial has applied for the trademark of CERTIFECT in the United States and it is a registered trademark in other countries. FRONTLINE Plus® is a registered trademark of Merial.

®Allergroom is a registered trademark of Virbac.

Table 1: Curative efficacy on *Dermacentor variabilis*

Untreated control			CERTIFECT™			K9 ADVANTIX®		
	Tick Thumb Count Day 1 24 hrs	Tick Removal Count Day 2 48 hrs		Tick Thumb Count Day 1 24 hrs	Tick Removal Count Day 2 48 hrs		Tick Thumb Count Day 1 24 hrs	Tick Removal Count Day 2 48 hrs
Geometric Mean	23.1	30.5	Geometric Mean	4.4	0	Geometric Mean	11.5	7.6
Std Dev	7.2	7.9	Std Dev	6.2	0	Std Dev	4.9	5.2
Min	9	17	Min	0	0	Min	5	3
Max	33	40	Max	8	0	Max	21	20
			Efficacy (%)	86.0	100.0	Efficacy (%)	50.0	75.0

parasites of dogs.¹ The control of tick and flea infestation is based on regular administration of ectoparasiticides. Pet owners may neglect applying ectoparasiticides to their pets on a monthly basis, which can result in increased ectoparasite infestations. Upon becoming aware of the presence of these more readily observed ticks attached to their animal, the sense of urgency in administering a treatment that has immediate effect is increased. With the increased consumer awareness of ticks being vectors of viruses, bacteria and parasites of dogs and humans, this sense of urgency increases. In order to address this need, the therapeutic efficacy of acaricidal products should be as fast as possible. For spot on formulations, two criteria are important: the efficacy of the active ingredients and the speed of diffusion onto the skin and rapid onset of activity.²

The topical formulation of imidacloprid 10% w/v + permethrin 50% w/v (K9 ADVANTIX®) provides tick efficacy to complement the insecticide only activity of imidacloprid. The combination of fipronil + amitraz + (S)-methoprene in a dual chamber pipette is a new topical formulation, CERTIFECT™, developed in order to increase significantly the speed of kill against ticks as a result of the potentiation provided by amitraz.^{1,3}

The FRONTLINE Plus® combination of fipronil and (S)-methoprene formulated for

topical application to dogs and cats provides a broad spectrum of activity against insects (including fleas, flea eggs, and flea larvae, and lice) and acarids (including ticks and other mites). The addition of amitraz to fipronil and (S)-methoprene potentiates the acaricidal effects of fipronil to significantly increase the speed of kill of ticks.^{1,3} Imidacloprid is an insecticide with no labelled activity against acarids. Thus, permethrin was added to imidacloprid to broaden the range of efficacy to include ticks.

In this study, we tested the curative efficacy of CERTIFECT™ and K9 ADVANTIX® on dogs having a mixed infestation with *Rhipicephalus sanguineus* and *Dermacentor variabilis*.

STUDY DESIGN

This study was a randomized, blinded efficacy study. The efficacy of the test product, CERTIFECT, was compared to a positive control, K9 ADVANTIX, and an untreated negative control. The experimental unit was the individual healthy dog which was identified, treated, and assessed for the study variables on an individual basis. The dogs had not been exposed to ectoparasiticides in the previous 3 months. From a group of 28 mixed-breed dogs, 24 dogs (15 males and 9 females) weighing 10.7 to 24.9 kg and ranging in age from 1.4 to 13.8 years of age were selected to be utilized in this study. Each dog was shampooed with a non-insec-

Table 2: Curative efficacy on *Rhipicephalus sanguineus*

Untreated control			CERTIFECT™			K9 ADVANTIX®		
	Tick Thumb Count Day 1 24 hrs	Tick Removal Count Day 2 48 hrs		Tick Thumb Count Day 1 24 hrs	Tick Removal Count Day 2 48 hrs		Tick Thumb Count Day 1 24 hrs	Tick Removal Count Day 2 48 hrs
Geometric Mean	22.8	29.2	Geometric Mean	4	0.3	Geometric Mean	12.6	7.1
Std Dev	6.4	9.8	Std Dev	3	0.7	Std Dev	7.3	5.5
Min	13	16	Min	2	0	Min	4	2
Max	33	43	Max	10	2	Max	26	19
			Efficacy (%)	82.4	99.1	Efficacy (%)	44.6	75.8

ticidal shampoo (Allergroom® Shampoo) for approximately 5 minutes on Day -10. Two 14 dog weight groups were formed on Day -5. Dogs in Weight Group 1 weighed 10.7 to 14.6 kg, and dogs in Weight Group 2 weighed 19.2 to 24.9 kg. A pre-treatment tick infestation was conducted on the 28 dogs to determine the susceptibility to infestation. The dogs were infested on the dorsal midline with 50 (±5) *R. sanguineus* on Day -7. The ticks were removed and counted on Day -5, and the one dog from Weight Group 1 and two dogs from Weight Group 2 with the lowest pre-qualification tick counts were removed from the pool of dogs to be used on the study.

One dog from Weight Group 1 was removed for health reasons. The remaining 24 dogs were ranked by decreasing body weight. The three dogs with the highest body weights formed Replicate 1, the next formed Replicate 2, and so on until all dogs were allocated. Within replicates, each dog was randomly allocated to one of the three treatment groups. The adult unfed *R. sanguineus* were obtained from Elward II Labs, and the adult unfed *D. variabilis* were obtained from the Oklahoma State University Tick Rearing Facility. These ticks were from strains that are not known to be resistant to any ectoparasiticide. Personnel conducting tick counts, performing daily health observations, or involved in the collection of other

study data were blinded to the treatment assignments of the dogs. *R. sanguineus* (50 + 5) was infested on Day -2, and *D. variabilis* (50 + 5) on Day -1 pre-treatment.

Each drug/formulation was applied once on Day 0. The dogs were weighed for dosage determinations per the Schedule of Operations. All dogs weighed that had weights that were not equivalent to a whole pound had their weight rounded up to then next whole pound.

Dogs in Treatment Group 1 Were Untreated.

Dogs in Treatment Group 2 were treated with the appropriate pipette size of CERTIFECT to deliver a minimum dose of 6.7 mg/kg of fipronil, 8 mg/kg of amitraz, and 6.03 mg/kg of (S)-methoprene on dogs. For treatment administration, the total volume was applied on two separate spots placed on the midline of the neck and at the base of the neck, between the shoulder blades.

Bodyweight Range	Pipette Size	Pipette Volume (mL) s
23 – 44 lbs (10.5 – 20 kg)	M	2.14
45 – 88 lbs (20.5 – 40 kg)	L	4.28

Dogs in Treatment Group 3 were treated with the appropriate pipette of K9 ADVANTIX, delivering a minimum dose of 10 mg/kg of imidacloprid and 50 mg/kg of perme-

thrin per dog. For treatment administration, the total volume was applied according to the package instructions by parting the hair and applying directly on the skin in 3 sites on dogs weighing less than 20 lbs and four sites for dogs weighting 21 lbs or over, along the midline from the shoulder blades to the base of the tail.

Bodyweight Range	Pipette Size	Pipette Volume (mL) s
21 – 55 lbs (10 – 25 kg)	Red	2.5

The ticks were thumb counted at 24 (+/- 2) hours and then removed and counted 48 (+/- 3) hours post-treatment. During the 24-hr and 48-hr tick counts, the ticks were categorized alive or dead; attached or unattached; and engorged or not. Infestation rates > 25 % in at least six controls were considered to be an adequate infestation.

DATA ANALYSIS

Total counts of adult ticks in categories 1 through 3 and 6 were transformed to the natural logarithm of (count +1) for calculation of geometric means by treatment group at each time point. Percent reduction from the negative control group (Treatment Group 1) mean was calculated for Treatment Groups 2 and 3, if applicable, at every post-treatment time point using the formula $[(C - T) / C] \times 100$, where C is the geometric mean for the

negative control group and T is the geometric mean for Treatment Group 2 or 3. Treatment Group 2 was compared to each of the other treatment groups (Treatment Groups 1 and 3) using Analysis of Variance on log count. All testing was be two-sided at the significance level $\alpha=0.05$.

ANIMAL MANAGEMENT

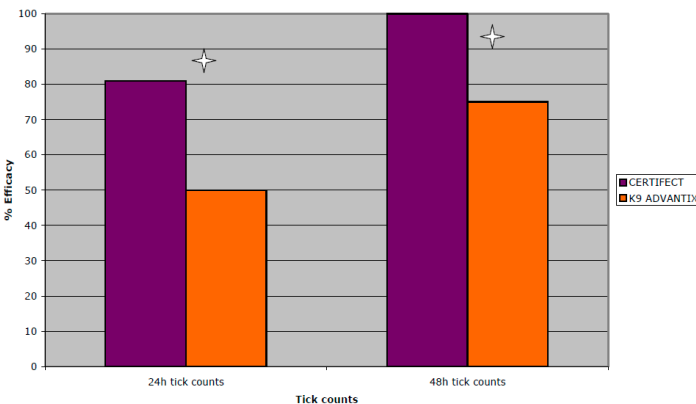
Animals were managed similarly and with due regard for their well-being. Animals were handled in compliance with the relevant Institutional Animal Care and Use Committee approvals. The animals were identified by means of unique tattooed number. The dogs utilized in the study were given a physical examination prior to being placed on study and were observed daily for health observations during the study.

RESULTS AND DISCUSSION

The tick counts and percent efficacy are shown in Tables 1 and 2 and Figures 1 and 2. There was >60% tick attachment rate for both *D. variabilis* and *R. sanguineus* in the control group. The post-treatment tick counts were lower at 24 hours than at 48 hours, which is due to of the lower sensitivity of thumb counting at 24 hours versus the tick removal and count method at 48 hours.

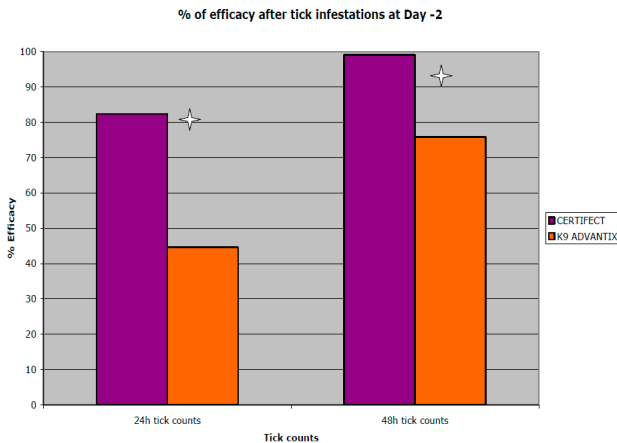
Fipronil-amitraz-(S)-methoprene demonstrated a significantly greater curative efficacy 24 hours post-treatment against *D. variabilis* (86%, $P=0.008$) and against *R. sanguineus* (82.4%, $P=0.007$) than imidacloprid-permethrin (50% and 44.6 %, respectively). At the 48 hours post-treatment tick count, CERTIFECT had a therapeutic efficacy of 100% against *D. variabilis* and 99.1% against *R. sanguineus*, while K9 ADVANTIX provided significantly lower ($P<0.0001$) efficacies of 75% and 75.8%, respectively.

Figure 1: Comparative efficacy of fipronil-amitraz-(S)-methoprene and imidacloprid-permethrin against *Dermacentor variabilis* 24 and 48 hours after treatment of tick-infested dogs



Significant difference at both time points at $p<0.05$

Figure 2: Comparative % of efficacy against *Rhipicephalus sanguineus*



Significant difference at both time points at $p < 0.05$

Fipronil-amitraz-(S)-methoprene provided a significantly greater ($P=0.008$) curative efficacy (84.3%) 24 hours post-treatment against the combined infestation with *D. variabilis* and *R. sanguineus* than imidacloprid-permethrin (47%) (Table 1). At the 48 hours post-treatment tick count, fipronil-amitraz-(S)-methoprene killed 99.5% of all ticks, while imidacloprid-permethrin provided significantly lower ($P < 0.0001$) efficacy (75.4%).

It has been previously demonstrated that the long lasting efficacy of fipronil+(S)-methoprene (FRONTLINE Plus) was significantly greater than that of imidacloprid+permethrin (K9 ADVANTIX) against *D. variabilis* and *D. reticulatus*.^{4,5,6,7} In these studies, tick counts were only conducted at 48 hours post-infestation and the efficacy was studied based on weekly infestations post-treatment, not on existing tick infestations. Other publications have reported the contact irritant effect obtained with permethrin formulations in trials using specific study designs of tick exposure to dogs.⁸ While not the purpose of this trial, it has been published elsewhere that the new combination of fipronil-amitraz-(S)-methoprene causes ticks to detach after product application.^{9, 10} This trial demonstrated a high curative efficacy of the

fipronil+amitraz+(S)-methoprene combination on existing tick infestations with *R. sanguineus* or *D. variabilis*, whereas the imidacloprid-permethrin formulation did not reach a 90% level of efficacy at 48 hours post-treatment. Moreover, the efficacy of the imidacloprid-permethrin formulation was less than 50% at 24 hour counts.

CERTIFECT when compared to K9 AVANTIX demonstrated a significantly ($P < 0.05$) greater efficacy in the reduction of *R. sanguineus* and *D. variabilis* ticks at 24 hours post-treatment, which is one of the attribute desired by pet owners seeing ticks on their animals.

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